BEFORE THE ENVIRONMENTAL PROTECTION AGENCY

Trichloroethylene; Regulation of Certain Uses under TSCA § 6(a)

[EPA-HQ-OPPT-2016-0163; FRL-9949-86]

Comments of the

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The Halogenated Solvents Industry Alliance, Inc. (HSIA) represents producers and users of trichloroethylene (TCE). We offer these comments on EPA's proposed rule banning manufacture of TCE for and use of TCE in aerosol degreasing and in spot cleaning by dry cleaning facilities. 81 Fed. Reg. 91592 (Dec. 16, 2016). This rule, proposed under § 6(a) of the Toxic Substances Control Act (TSCA), is based on a Work Plan Assessment of TCE completed by EPA in June 2014. TSCA was amended in June 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act ("Lautenberg Act").

HSIA urges EPA to withdraw the proposed rule, which is based on a very deficient risk assessment.. While EPA is authorized under TSCA § 26(l)(4) to propose a § 6 rule based on a risk assessment completed before TSCA was revised, there is no requirement or deadline for it to do so. The situation is very different for the ten priority compounds designated by EPA under TSCA § 6(b)(2)(A) in December 2016.[1] For these ten designated pollutants, TSCA establishes deadlines for risk assessments and rulemakings. TCE is one of the ten priority compounds, and the better course would be to assess the risks from spot cleaning and aerosol degreasing as part of the required upcoming TCE assessment.

These comments address the following subjects, among others, in detail:

- TSCA § 26(l)(4) requires, for a rule based on a risk assessment completed before TSCA was revised, that the rule must be consistent with "the scope of the completed risk assessment for the chemical substance and consistent with other applicable requirements of § 6." "Although the use of TCE as a solvent degreaser at large commercial/industrial operations" was "not considered in this assessment," EPA nevertheless would prohibit all "commercial use of TCE in aerosol degreasing products," regardless of the size of the facility. This is plainly outside "the scope of the completed risk assessment."

- Further, the TCE Work Plan Assessment does not comply with the requirements of TSCA § 6(b)(4)(F) or TSCA § 26(h) and (i), which are expressly applicable to any EPA "decision based on science" under TSCA § 6. The disparity between the completed risk assessments and the "applicable requirements of § 6" is obvious from even a cursory review of the procedures for risk evaluation under the amended TSCA proposed by EPA earlier this year.

- The Work Plan Assessment expressly relied on hazard values derived directly from a University of Arizona study to estimate non-cancer risk. Several other studies, including two GLP-compliant studies conducted under EPA and OECD guidelines, have been unable to reproduce the effect seen in the Arizona study. The Arizona study has been heavily criticized in the published literature, its results have not been replicated by any other laboratory, and other regulatory authorities (including the California EPA) have rejected the study as deficient.

- Equally, the Work Plan Assessment relies on qualitative and quantitative estimates of cancer risk that are not realistic or justified by any underlying science. EPA estimates a baseline cancer risk from chronic occupational spot cleaning exposures of 1 in 10. Cancer incidence of this magnitude could not go unnoticed, and indeed EPA's estimate is belied by available epidemiology studies of dry cleaning workers which show no such risk. Indeed, two recent large Nordic epidemiological studies, both of which had extensive follow-up of the cohorts, have failed to find an association between TCE and kidney cancer, and these are not addressed in the Work Plan Assessment. Further, EPA's development of a potency factor based on Charbotel *et al.* (2006) directly contravenes the advice EPA received from the National Academy of Sciences.

[1] 81 Fed. Reg. 91927 (Dec.19, 2016).

- On the exposure side, for spot cleaning EPA relied solely on a 2007 California study, which it recognized may not be representative of US dry cleaning facilities. For aerosol degreasing EPA provided no emissions or monitoring data – thus these are hypothetical exposures. Moreover, the draft TCE assessment, entitled "Degreaser and Arts/Crafts Uses," did not address spot cleaning (except to say that none of those sold to consumers contained TCE), but the final Work Plan Assessment is entitled "Degreasing, *Spot Cleaning* and Arts & Crafts Uses" and includes commercial use of TCE as a spotting agent at dry cleaning facilities.

- Because there was no notice that EPA was addressing spot cleaning, there was no participation by dry cleaner representatives and no peer review of the spot cleaning assessment. Moreover, there was no Small Business Advocacy Review, even though spot cleaning is done by dry cleaners which are virtually all small entities. It is not credible that EPA could certify that the rule would not have a significant economic impact on a substantial number of small entities (SISNOSE), where the dry cleaning industry estimates that 60-90% of retail dry cleaners routinely use TCE on the spotting board (14,130 – 21,195 small businesses) and projects that such a ban will cost 4-5% of gross sales, far more than the 1-3% impact considered SISNOSE.

- Peer review of the draft Work Plan Assessment was scathing. Reliance on the unreproducible Arizona study was harshly criticized. The Chair of the panel noted that it was a screening level assessment, not suitable for use in regulation: "*the Agency acted prematurely in issuing this (screening level)* assessment for public comment. . . . After listening carefully to the comments and contributions from the other members of the Panel, I have concluded that there would *little benefit in revising this draft screening assessment.*"[2] Yet EPA claims the peer review was supportive.

- EPA's determination that TCE use in spot cleaning and aerosol degreasing poses an "unreasonable risk" is based on its assessment of risks to workers. It is clear, however, that TSCA is to be used only when other statutes fail to provide a remedy for unreasonable risks. Worker health and safety fall under the jurisdiction of the Occupational Safety and Health Administration (OSHA), and use of TCE in spot cleaning and spray degreasing is already adequately regulated under the Occupational Safety and Health Act. Congress cannot have meant, in enacting "gap-filling" legislation, to open the door to EPA assuming all authority over the use of hazardous substances in the workplace.

I. Failure of Work Plan Assessment to Comply with TSCA §§ 6, 26

A. Applicable Requirements of TSCA §§ 6, 26

Although the Lautenberg Act made significant changes to TSCA to ensure that EPA would employ the "best available science" in its risk assessments, EPA proposes to rely on a remarkably sketchy and inadequate assessment in its inaugural rulemaking under TSCA § 6. TSCA § 6(b)(4)(F), as revised by the Lautenberg Act, requires that EPA's risk evaluations must, among other things:

- "integrate and assess available information on hazards and exposures for the conditions of use of the chemical substance, including information that is relevant to specific risks of injury to health or the environment and information on potentially exposed or susceptible subpopulations identified as relevant by the Administrator;"

[2] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

- "take into account, where relevant, the likely duration, intensity, frequency, and number of exposures under the conditions of use of the chemical substance;" and

- "describe the weight of the scientific evidence for the identified hazard and exposure."

New TSCA § 26(h) requires that, in carrying out § 6, "to the extent that the Administrator makes a decision based on science, the Administrator shall use scientific information, technical procedures, measures, methods, protocols, methodologies, or models, employed in a manner consistent with the best available science, and shall consider as applicable—

> (1) the extent to which the scientific information, technical procedures, measures, methods, protocols, methodologies, or models employed to generate the information are reasonable for and consistent with the intended use of the information;
>
> (2) the extent to which the information is relevant for the Administrator's use in making a decision about a chemical substance or mixture;
>
> (3) the degree of clarity and completeness with which the data, assumptions, methods, quality assurance, and analyses employed to generate the information are documented;
>
> (4) the extent to which the variability and uncertainty in the information, or in the procedures, measures, methods, protocols, methodologies, or models, are evaluated and characterized; and
>
> (5) the extent of independent verification or peer review of the information or of the procedures, measures, methods, protocols, methodologies, or models."

With regard to risk assessments completed prior to passage of the Lautenberg Act, including that for TCE, TSCA § 26(l)(4) provides that "the Administrator may publish proposed and final rules under section 6(a) that are consistent with the scope of the completed risk assessment for the chemical substance and consistent with other applicable requirements of section 6." Thus, EPA may base regulation on the pre-enactment risk assessments only to the extent that they comply with the substantive requirements above.

Regrettably, the proposal to ban TCE in aerosol degreasing addresses a broader scope of uses than considered in the Work Plan Assessment. The scope of that assessment is clear: "although the use of TCE as a solvent degreaser at large commercial/industrial operations is expected to be frequent and the concentration of TCE high, human exposures in these settings are expected to be monitored and controlled by Occupational Safety & Health Administration (OSHA); thus, this use is also not considered in this assessment" (p. 27). The Assessment is focused solely on exposure from TCE use as a solvent degreaser in small commercial settings and by consumers.[3] The proposed ban, however, recognizes no such limitation. It would prohibit commercial use of TCE for general aerosol degreasing, as well as its manufacture, processing, and distribution in commerce for this use. Because the proposed rule would ban uses beyond the scope of the underlying Work Plan Assessment, it is not "consistent with the scope of the completed risk assessment" and therefore does not comply with TSCA § 26(l)(4).

[3] *See* Work Plan Assessment at Table 1-1.

Further, the proposed rule does not comply with the requirements of TSCA § 6(b)(4)(F) or TSCA § 26(h) and (i), which are expressly applicable to any EPA "decision based on science" under TSCA § 6. The disparity between the completed TCE Work Plan Assessment and the "applicable requirements of § 6" is obvious from a review of the procedures for risk evaluation under the amended TSCA proposed by EPA earlier this year.[4]

B. Deficiencies of Principal Non-Cancer Study

1. Not Reproducible

The Work Plan Assessment expressly relies on hazard values derived directly from a single academic study to estimate non-cancer risk.[5] Specifically, it states (p. 104):

> "The acute inhalation risk assessment used developmental toxicity data to evaluate the acute risks for the TSCA TCE use scenarios. As indicated previously, EPA's policy supports the use of developmental studies to evaluate the risks of acute exposures. This policy is based on the presumption that a single exposure of a chemical at a critical window of fetal development, as in the case of cardiac development, may produce adverse developmental effects (EPA, 1991).
>
> "After evaluating the developmental toxicity literature of TCE, the TCE IRIS assessment concluded that the fetal heart malformations are the most sensitive developmental toxicity endpoint associated with TCE exposure (EPA, 2011e). Thus, EPA/OPPT based its acute risk assessment on the most health protective endpoint (i.e., fetal cardiac malformations; Johnson et al., 2003) representing the most sensitive human population (i.e., adult women of childbearing age and fetus >16 yrs).
>
> "The acute risk assessment used the PBPK-derived hazard values (HEC_{50}, HEC_{95}, or HEC_{99}) from Johnson et al. (2003) developmental study for each degreaser and spot cleaner use scenario. . . . These extremely low values result in margin of exposure ("MOE") values below 10 for almost all the occupational and residential exposure scenarios examined."

A single flawed study should not be the basis for the toxicological value that serves as the basis for regulation. Several other studies, including three GLP-compliant studies conducted under EPA guidelines to support pesticide registration (40 CFR § 870.3700) and Organization for Economic Coordination & Development ("OECD") guidelines (414) have been unable to reproduce the effect seen by Johnson *et al.* (2003).

Johnson *et al.* (2003) reported cardiac effects in rats from research carried out at the University of Arizona and originally published ten years earlier by the same authors.[6] In the earlier-published study, there was no difference in the percentage of cardiac abnormalities in rats dosed during both pre-mating

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[4] 82 Fed. Reg. 7562 (Jan. 19, 2017).

[5] Johnson PD, *et al.*, Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat, Environ Health Perspect. 111:289-92 (2003).

[6] Dawson, B, *et al.*, Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water, J. Am. Coll. Cardiol. 21: 1466-72 (1993).

and pregnancy at drinking water exposures of 1100 ppm (9.2%) and 1.5 ppm (8.2%), even though there was a 733-fold difference in the concentrations. The authors reported that the effects seen at these exposures were statistically higher than the percent abnormalities in controls (3%). For animals dosed only during the pregnancy period, the abnormalities in rats dosed at 1100 ppm (10.4%) were statistically higher than at 1.5 ppm (5.5%), but those dosed at 1.5 ppm were not statistically different from the controls. Thus, no meaningful dose-response relationship was observed in either treatment group. Johnson *et al.* republished in 2003 data from the 1.5 and 1100 ppm dose groups published by Dawson *et al.* in 1993 and pooled control data from other studies, an inappropriate statistical practice, to conclude that rats exposed to levels of TCE greater than 250 ppb during pregnancy have increased incidences of cardiac malformations in their fetuses.

2. Criticism in Literature and by Other Regulators

Johnson *et al.* (2003) has been heavily criticized in the published literature.[7] Indeed, its predecessor study was expressly rejected as the basis for MRLs by the Agency for Toxic Substances & Disease Registry (ATSDR) in its last TCE Toxicological Profile Update.[8] Moreover, the Johnson *et al.* (2003) findings were not reproduced in a study designed to detect cardiac malformations; this despite employing an improved method for assessing cardiac defects and the participation of Dr. Johnson herself.[9] No increase in cardiac malformations was observed in the second guideline study,[10] despite high inhalation doses and techniques capable of detecting most of the malformation types reported by Johnson *et al.* (2003). The dose-response relationship reported in Johnson *et al.* (2003) for doses spanning an extreme range of experimental dose levels is considered by many to be improbable, and has not been replicated by any other laboratory.[11]

Even the California Office of Environmental Health Hazard Assessment (OEHHA) rejected the study as deficient:

> "Johnson et al. (2003) reported a dose-related increased incidence of abnormal hearts in offspring of Sprague Dawley rats treated during pregnancy with 0, 2.5 ppb, 250 ppb, 1.5 ppm, and 1,100 ppm TCE in drinking water (0, 0.00045, 0.048, 0.218, and 128.52 mg/kg-day, respectively). The NOAEL for the Johnson study was reported to be 2.5 ppb (0.00045 mg/kg-day) in this short exposure (22 days) study. The percentage of abnormal hearts in the control group was 2.2 percent, and in the treated groups was 0 percent (low dose), 4.5 percent (mid dose 1), 5.0 percent (mid dose 2), and 10.5 percent (high dose).

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[7] Hardin, B, *et al.*, Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004); Watson, R., *et al.*, Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature, Repro. Toxicol. 21: 117-47 (2006).

[8] ATSDR concluded that "[t]he study is limited in that only two widely spaced exposure concentrations were used and that a significant dose-response was not observed for several exposure scenarios." Toxicological Profile for Trichloroethylene Update (September 1997), at 88.

[9] Fisher, J, *et al.*, Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development? Int. J. Toxicol. 20: 257-67 (2001).

[10] Carney, E, *et al.*, Developmental toxicity studies in Crl:Cd (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene. Birth Defects Research (Part B) 77: 405-412 (2006).

[11] "Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a 'specific' cardiac teratogen." Hardin, B, *et al.*, Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004).

> The number of litters with fetuses with abnormal hearts was 16.4 percent, 0 percent, 44 percent, 38 percent, and 67 percent for the control, low, mid 1, mid 2, and high dose, respectively. The reported NOAEL is separated by 100-fold from the next higher dose level. The data for this study were not used to calculate a public-health protective concentration since a meaningful or interpretable dose-response relationship was not observed. *These results are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits: The other studies did not find adverse effects on fertility or embryonic development, aside from those associated with maternal toxicity (Hardin et al., 2004).*"[12]

3. Reservations of EPA Scientific Staff

Remarkably, an EPA staff review that was placed in the docket for the Work Plan Assessment reflects similar concerns. First, one staff member dissented over relying at all on the Arizona study:

> "The rodent developmental toxicology studies conducted by Dawson et al. (1993), Johnson et al. (2003), and Johnson et al. (1998) that have reported cardiac defects resulting from TCE (and metabolite) drinking water exposures have study design and reporting limitations. Additionally, two good quality (GLP) inhalation and gavage rodent studies conducted in other laboratories, Carney et al. (2006) and Fisher et al. (2001), respectively, have not detected cardiac defects. These limitations and uncertainties were the basis of the single dissenting opinion of a team member regarding whether the database supports a conclusion that TCE exposures during development are likely to cause cardiac defects."[13]

Second, even the EPA staff that agreed with use of the study had little confidence that it supported the dose-response assessment:

> "[A] majority of the team members agreed that the Johnson et al. (2003) study was suitable for use in deriving a point of departure. However, confidence of team members in the dose response evaluation of the cardiac defect data from the Johnson et al. (2003) study was characterized as between 'low' and 'medium' (with 7 of 11 team members rating confidence as 'low' and four team members rating confidence as 'low to medium')."[14]

It is surprising that EPA would consider use of a dose-response value for regulation from a study in which seven of its own scientists expressed "low" confidence, and in which the other four could muster no more than "low to medium" confidence. The same report notes: "In conclusion, there has not been a confirmation of the results of the Johnson et al. (2003) and Dawson et al. (1993) studies by another laboratory, but there has also not been a repeat of the exact same study design that would corroborate or refute their findings."

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[12] California EPA Public Health Goal for Trichloroethylene in Drinking Water (July 2009), at 21 (emphasis added).

[13] TCE Developmental Cardiac Toxicity Assessment Update (available at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2012-0723-0045).

[14] *Id.*

4. EPA's Dose-Response Evaluation using Johnson *et al.* (2003) Is Inappropriate

The TCE Work Plan Assessment relies on the prior IRIS Assessment's evaluation of the relationship between TCE exposure dose and the development of cardiac defects, as described in Johnson *et al.* (2003). Ignoring for the moment the myriad of methodological deficiencies in the paper, a closer look at EPA's evaluation of that dose-response relationship in generating a point of departure (POD) raises several concerns. The importance of this activity cannot be overstated, as according to a paper published by the authors of the IRIS Assessment, Johnson *et al.* (2003) represents "the only available study potentially useable for dose-response analysis of fetal cardiac defects."[15]

In discussing the dose-response evaluation, Makris *et al.* (2016) further state that "[g]iven the uncertainties in the dose-response analysis related to the nature of the data, the confidence in the POD based on Johnson *et al.* (2003) has limitations. Overall, however, the POD derived in the 2011 TCE assessment (U.S. EPA, 2011), which used an approach consistent with standard U.S. EPA dose-response practices, remains a reasonable choice." It should be noted that, in order to achieve a better model fit in its derivation of a POD, EPA dropped the highest exposure dose from Johnson *et al.* (2003). With already questionable data, and no expectation that the highest dose of TCE would result in a diminished response, that decision should be reconsidered.

Makris *et al.* (2016) describe additional dose-response analyses performed to characterize the uncertainty in the POD. In summarizing the results of this analysis, they state that "[a]lternative PODs were derived based on use of alternative models, alternative BMR levels, or alternative procedures (such as LOAEL/NOAEL approach), each with different strengths and limitations. These alternatives were within *about an order of magnitude of the POD derived in the 2011 TCE assessment*" (emphasis added). This level of uncertainty in modeling the POD when combined with the uncertainty in the PBPK modeling (discussed elsewhere) and the overall poor quality of the underlying developmental toxicity study provide little confidence in the resulting non-cancer toxicological value in the Work Plan Assessment that drives the proposed regulation.

5. Reliance on Johnson *et al.* (2003) Is Inconsistent with Use of Best Available Science

All acute inhalation exposures in the TCE Work Plan Assessment were measured against potential developmental toxicity endpoints based solely on EPA's IRIS evaluation of Johnson *et al.* (2003). When HSIA requested access to the data used by EPA in its evaluation of the dose-response relationship between TCE exposure and cardiac defects reported in Johnson *et al.* (2003), the Agency provided the spreadsheet, referenced as Johnson (2009) (HERO ID 783484) in the 2011 IRIS Assessment, and indicated that was the entirety of the data evaluated. Examination of that spreadsheet reveals an absence of certain critical information, including, most importantly, dates for any of the individual treatment/control animals.

Acknowledging the documented deficiencies in their paper (and the data provided to EPA), the authors published an erratum aimed at updating the public record regarding methodological issues for Johnson *et al.* (2003).[16] According to Makris *et al.* (2016):

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[15] Makris SL, Scott CS, Fox J, *et al.*, Systematic evaluation of the potential effects of trichloroethylene exposure on cardiac development. Repro Toxicol (2016); http://dx.doi.org/10.1016/j.reprotox.2016.08.014

[16] Johnson PD, Goldberg SJ, Mays MZ, Dawson BV, Erratum: Erratum for Johnson *et al.* [Environ Health Perspect 113: A18 (2005)]; Environ Health Perspect 122: A94 (2014); http://dx.doi.org/10.1289/ehp.122-A94

> "some study reporting and methodological details remain unknown, *e.g.*, the precise dates that each individual control animal was on study, maternal body weight/food consumption and clinical observation data, and the detailed results of analytical chemistry testing for dose concentration. Additional possible sources of uncertainty identified for these studies include that the research was conducted over a 6-yr period, that combined control data were used for comparison to treated groups, and that exposure characterization may be imprecise because tap (rather than distilled) drinking water was used in the Dawson *et al.* (1993) study and because TCE intake values were derived from water consumption measures of group-housed animals."

HSIA submits that the information contained in the above paragraph alone should disqualify Johnson *et al.* (2003) as "best available science" as required under EPA's proposed procedures for chemical risk evaluation under TSCA as amended.[17]

6. Failure to Conform to EPA Guidelines for Developmental Toxicity Risk Assessment

EPA's Guidelines for Developmental Toxicity Risk Assessment establish the framework for evaluation of developmental toxicity risk on a case-by-case basis.[18] Under these Guidelines, "[i]f data are considered *sufficient* for risk assessment, an oral or dermal reference dose for developmental toxicity (RfD_{DT}) or an inhalation reference concentration for developmental toxicity (RfC_{DT}) is then derived for comparison with human exposure estimates" (emphasis added).

In defining sufficiency, the Guidelines state: "In the case of animal data, agents that have been tested adequately in laboratory animals *according to current test guidelines* generally would be included in the "Sufficient Experimental Animal Evidence/Limited Human Data" category (emphasis added)." Where, as here, the "database on a particular agent includes less than the minimum sufficient evidence (as defined in the 'Insufficient Evidence' category) necessary for a risk assessment, but some data are available, this information could be used to determine the need for additional testing. . . . In some cases, a database may contain conflicting data. In these instances, the risk assessor must consider each study's strengths and weaknesses within the context of the overall database in an attempt to define the strength of evidence of the database for assessing the potential for developmental toxicity."

Given the demonstrated shortcomings of Johnson *et al.* (2003), which was not conducted to EPA test guidelines, and the availability to EPA of three guideline studies, we submit that the Guidelines for Developmental Toxicity Risk Assessment and TSCA §§ 6 and 26 require a weight of evidence evaluation of the database before EPA relies on Johnson *et al.* (2003) for regulatory purposes.

7. New Relevant Information

A third guideline study of TCE developmental toxicity has been sponsored by HSIA. The study was designed with a focus on cardiac abnormalities and included toxicokinetic measures to enable comparison with the earlier studies. It was intended to fill the remaining gap for a guideline study by the drinking water route, the same exposure route as Johnson *et al.* (2003). Regrettably, although the in-life

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[17] 82 Fed. Reg. 7562 (Jan. 19, 2017).

[18] 56 Fed. Reg. 63798 (December 5, 1991).

portion of the study was conducted during October and November, 2016, the concentrations of TCE measured in the drinking water solutions were found to be below the acceptable target range of 100% ± 10%, invalidating the study. The laboratory is conducting additional studies to identify the source of the deviations and the study will be rerun as soon as the dosing methodological issues are resolved and scheduling permits. A statement to this effect is attached as Appendix 1.

C. Deficiencies of Cancer Risk Assessment

1. Erroneous Characterization of TCE as "Carcinogenic to Humans"

While acute risks of developmental toxicity are characterized by EPA as of the greatest concern, the Work Plan Assessment also concludes that all but one of the degreaser exposure scenarios exceeded all the target cancer levels. The discussion of carcinogenicity in the Work Plan Assessment suffers from slavish reliance on EPA's earlier assessment of TCE under its Integrated Risk Information System.[19] The IRIS Assessment classifies TCE as "Carcinogenic to Humans." It fails to discuss (or even to recognize) that such classification is inconsistent with a definitive report by the National Academy of Sciences, discussed below.[20] We briefly address below how the epidemiological data on TCE do not meet the threshold for classification as "Carcinogenic to Humans."

a. Guidelines for Carcinogen Risk Assessment

EPA's 2005 Guidelines for Carcinogen Risk Assessment provide the following descriptors as to the weight of evidence for carcinogenicity:

- Carcinogenic to humans,
- Likely to be carcinogenic to humans,
- Suggestive evidence of carcinogenicity,
- Inadequate information to assess carcinogenic potential, and
- Not likely to be carcinogenic to humans.[21]

According to the Guidelines, "carcinogenic to humans" means the following:

"This descriptor indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- "This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

[19] "Toxicological Review of Trichloroethylene (CAS No. 79-01-6) in Support of Summary Information on the Integrated Risk Information System (IRIS)" ("IRIS Assessment")

[20] National Research Council. Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects (2009) (hereinafter "Camp Lejeune report").

[21] 70 Fed. Reg. 17766-817 (April 7, 2005).

- "Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* of the following conditions are met: (a) There is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, *and* (b) there is extensive evidence of carcinogenicity in animals, *and* (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, *and* (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on mode of action and also an indication of the relative weight that each source of information carries, *e.g.*, based on human information, based on limited human and extensive animal experiments."

According to the Guidelines, the descriptor "likely to be carcinogenic to humans":

"is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor 'Carcinogenic to Humans.' Adequate evidence consistent with this descriptor covers a broad spectrum. . . . Supporting data for this descriptor may include:

"An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer:

- "An agent that has tested positive in animal experiments in more than one species, sex, strain, site or exposure route, with or without evidence of carcinogenicity in humans;

- "A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy or an early age at onset;

- "A rare animal tumor response in a single experiment that is assumed to be relevant to humans; or

- "A positive tumor study that is strengthened by other lines of evidence."

According to the Guidelines, the descriptor "suggestive evidence of carcinogenicity":

"is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples include:

- "A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans;'

- "A small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;

- "Evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence; or

- "A statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

b. Application of the Guidelines to TCE

In considering the data in the context of applying the "Carcinogenic to Humans" descriptor, one first considers the weight of the epidemiological evidence. We judge the epidemiologic evidence to be neither "convincing" nor "strong," two key terms in the Guidelines. This judgment is based on four recent reviews and meta-analyses of occupational TCE exposures and cancer as well as other reviews of this literature.[22] The recent review and meta-analysis by Kelsh *et al.* focuses on occupational TCE exposure and kidney cancer, and includes the Charbotel *et al.* study that is emphasized in the IRIS and Work Plan Assessments.[23] Both the EPA meta-analysis and the Kelsh *et al.* meta-analysis of the TCE kidney cancer epidemiologic literature produced similar summary results. However in Kelsh *et al.* the limitations of this body of research, namely exposure assessment limitations, potential unmeasured confounding, potential selection biases, and inconsistent findings across groups of studies, did not allow for a conclusion that there is sufficient evidence of a causal association, despite a modest overall association.

There are reasonably well-designed and well-conducted epidemiologic studies that report no association between TCE and cancer, some reasonably well-designed and conducted studies that did report associations between TCE and cancer, and finally some relatively poorly designed studies reporting both positive and negative findings. Overall, the summary relative risks or odds ratios in the meta-analysis studies (EPA or published meta-analyses) generally ranged between 1.2 and 1.4. The draft assessment refers to these associations as "small," a term not

[22] Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukaemia, Occup Med (Lond) 56:485–493 (2006); Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and liver cancer, Int Arch Occup Environ Health 81(2):127–43 (2007); Mandel, J, *et al.*, Occupational trichloroethylene exposure and non-Hodgkin's lymphoma: a meta-analysis and review, Occup Environ Med 63:597–607 (2006); Kelsh, M, *et al.*, Occupational trichloroethylene exposure and kidney cancer: a meta-analysis, Epidemiology 21(1): 95-102 (January 2010).

[23] Charbotel, B, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part II: Epidemiological aspects, Ann Occup Hyg 50(8):777–787 (2006).

typically consistent with "convincing" and "strong." Weak or small associations may be more likely to be influenced by or be the result of confounding or bias. Smoking and body mass index are well-established risk factors for kidney cancer, and smoking and alcohol are risk factors for liver cancer, yet the potential impact of these factors on the meta-analysis associations was not fully considered. There were suggestions that these factors may have impacted findings (*e.g.*, in the large Danish cohort study of TCE exposed workers, the researchers noted that smoking was more prevalent among the TCE exposed populations, however little empirical data were provided). In addition, co-linearity of occupational exposures (*i.e.*, TCE exposure correlated with chemical and/or other exposures) may make it difficult to isolate potential effects of TCE from those of other exposures within a given study, and hinder interpretation across studies. For example, although Charbotel *et al.* reported potential exposure response trends, while controlling for many confounders of concern (which strengthens the weight of evidence), they also reported attenuated associations for cumulative TCE exposure after adjustment for exposure to cutting fluids and other petroleum oils (weakening the weight of the evidence). This study is also limited due to other potential study design considerations such as selection bias, self reporting of work histories, and residual confounding.

When examining the data for TCE and non-Hodgkin lymphoma, kidney cancer, and liver cancer, associations were inconsistent across occupational groups (summary results differed between aerospace/aircraft worker cohorts compared with workers from other industries), study design, location of the study, quality of exposure assessment (*e.g.*, evaluating studies that relied upon biomonitoring to estimate exposure *vs.* semi-quantitative estimates *vs.* self-report, etc.), and by incidence *vs.* mortality endpoints. Although EPA examined high dose categories, it did not evaluate any potential dose-response relationships across the epidemiologic studies (except for Charbotel *et al.*). Reviews of the epidemiologic data reported in various studies for different exposure levels (*e.g.*, cumulative exposure and duration of exposure metrics) did not find consistent dose-response associations between TCE and the three cancer sites under review.[24] An established dose-response trend is one of the more important factors when making assessments of causation in epidemiologic literature. Thus, based on an overall weight of evidence analysis of the epidemiologic research, these data do not support the conclusion that there is "strong" or "convincing" evidence of a causal association between human exposure and cancer.

EPA's Guidelines also state that a chemical may be described as "Carcinogenic to Humans" with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence, all of which must be met. One of these lines of evidence is "extensive evidence of carcinogenicity in animals." Therefore, we must briefly evaluate the animal data.

The criteria that have to be met for animal data to support a "carcinogenic to humans" classification are stated in a sequential manner with an emphasized requirement that all criteria have to be met. Since the Guidelines consider this to be an "exceptional" route to a "carcinogenic to humans" classification, we would expect rigor to have been applied in assessing animal data against the criteria. This simply was not done.

Of the four primary tissues that EPA evaluated for carcinogenicity, only one or perhaps two rise to the level of biological significance. Discussion of the remaining tumor types appears to presuppose

[24] Mandel, J, *et al.*, Occupational trichloroethylene exposure and non-Hodgkin's lymphoma: a meta-analysis and review, Occup Environ Med 63:597–607 (2006); Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and liver cancer, Int Arch Occup Environ Health 81(2):127–43 (2007); Kelsh, M, *et al.*, Occupational trichloroethylene exposure and kidney cancer: a meta-analysis, Epidemiology 21(1): 95-102 (January 2010).

that TCE is carcinogenic. The resulting discussion appears then to overly discount negative data, of which there are many, and to highlight marginal findings. The text does not appear to be a dispassionate rendering of the available data. Specifically, EPA's conclusion that kidney cancer is evident in rats rests on *one* statistically significant finding in over 70 dose/tumor endpoint comparisons and references to exceedances of historical control values.[25] Using a 0.05 p-value for statistical significance, a frequency of 1 or even several statistically or biologically significant events is expected in such a large number of dosed/tumor groups. EPA's overall conclusion based on these flawed studies cannot be that TCE is a known kidney tumorigen. The best that can be said is that the data are inconsistent. Certainly they do not meet the criterion of "extensive evidence of carcinogenicity in animals." Several marginal findings do not constitute "extensive evidence."

For all these reasons, EPA's classification of TCE as "Carcinogenic to Humans" is not supported by the evidence and cannot be justified under the 2005 Guidelines.[26]

c. EPA's Position that there is 'Convincing Evidence' that TCE Is Carcinogenic to Humans is Inconsistent with National Academy of Sciences Conclusion of only 'Limited or Suggestive Evidence'

The IRIS Assessment states that "TCE is characterized as 'carcinogenic to humans' by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer."

Box 2 of the Academy's Camp Lejeune report, attached as Appendix 3, categorizes every cancer outcome reviewed in relation to exposure to TCE, the dry cleaning solvent perchloroethylene, or a mixture of the two. The categories are taken directly from a respected Institute of Medicine (IOM) report.[27] These categories are "sufficient evidence of a causal relationship," "sufficient evidence of an association," "limited or suggestive evidence of an association," "inadequate evidence to determine an association," and "limited or suggestive evidence of no association," all as defined in Box 1, also attached.

Looking at Box 2, evidence considered by EPA to be "convincing evidence of a causal association between TCE exposure in humans and kidney cancer" would seem to be considered "sufficient evidence of a causal relationship." Yet the Academy found no outcomes in that category. It would at least be "sufficient evidence of an association." Again, the Academy found no outcomes in that category. Only in the third category, "limited or suggestive evidence of an association," does one find kidney or any other cancer outcome associated with TCE.

"Limited evidence of an association" is far from "convincing evidence of causation." One would expect at the least a detailed explanation of EPA's very different conclusion. Although the 2009 Camp Lejeune study was already published, and indeed is cited in the references, there is no mention of it in the text of the IRIS Assessment, even though the previous draft had just been the subject of a multi-year review by the Academy.

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[25] And that bioassay is from a laboratory whose studies EPA has reviewed and declined to rely upon in other assessments.

[26] Further commentary to this effect, provided by a distinguished group of consultants in connection with the TCE IRIS Assessment but not addressed by EPA, is attached as Appendix 2.

[27] Institute of Medicine, Gulf War and Health, Vol. 2, Insecticides and Solvents (National Academies Press) (2003).

The Camp Lejeune committee began with a comprehensive review of the epidemiology studies of the two solvents by the IOM for its Gulf War Report. They then identified new studies published from 2003 to 2008 and considered whether these changed the conclusions in the IOM report. In the case of TCE and kidney cancer, this was the case. The Camp Lejeune committee considered six new cohort studies and two case-control studies (including Charbotel *et al.*). They concluded that several of these studies reported an increased risk of kidney cancer, but observed that the results were often based on a relatively small number of exposed persons and varied quality of exposure data and methodology. Given these data, the committee raised the classification for TCE to match the IOM conclusion of "limited" evidence for perchloroethylene.

EPA, on the other hand, offered the summary conclusion of convincing human evidence, based on the "consistency" of increased kidney cancer across the different studies. The authors of these studies, however, do not agree with EPA's characterization of them. For example, the authors of Charbotel *et al.*, the study EPA finds most compelling, state that the "study suggests an association between exposures to high levels of TCE and increased risk of [renal cell carcinoma]. Further epidemiological studies are necessary to analyze the effect of lower levels of exposure."

Given the flaws in the IRIS Assessment, and the very different conclusion reached by the Academy in its Camp Lejeune report on the same body of data, the Work Plan Assessment should not rely on the IRIS Assessment's classification of TCE as "Carcinogenic to Humans."

2. EPA Should Reassess Available Cancer Epidemiology Data, Given Publication of More Recent and Larger Studies on Worker Populations

The observation of an elevated but weak kidney cancer association reported by Charbotel *et al.* (2006)[28] contrasts with other occupational studies which did not find an elevation in kidney cancer in industries using TCE as a metal degreaser, *e.g.*, aircraft manufacturing, metal cleaning, etc., where exposures may be higher than for screw cutters. Lipworth and coworkers (2011) found no evidence of increased kidney cancer in a large worker cohort with multiple decades of TCE exposure and extended cancer follow-up evaluations.[29] The aircraft manufacturing study involved a total cohort of 77,943 workers, of which 5,443 were identified as exposed to TCE. The study involved evaluations from 1960 through 2008, at which time 34,248 workers had died. Approximately 30% of the workers were hired before 1960 (60% born before 1940), 52% terminated employment by 1980, and approximately a third of the workers were employed for more than 20 years. The standardized incidence ratio (SIR) for kidney cancer in the TCE-exposed workers was reported as 0.66 (CI 95%: 0.38-1.07). This value for the SIR indicates that these workers were potentially less likely to get kidney cancer than the normal population (or at least had the same rate as the normal population – SIR of 1).

More recently, two large Nordic country epidemiological studies, both of which had extensive follow-up of the cohorts, have likewise failed to find an association between TCE and kidney cancer. An SIR of 1.01 (0.70-1.42) was found by Hansen *et al.* (2013) for kidney cancer based on 32 cases out of a total of 997 cancer cases in a cohort of 5,553 workers in Finland, Sweden, and Denmark, indicating that

[28] Charbotel, B, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part II: Epidemiological aspects, Ann Occup Hyg 50(8):777–787 (2006).

[29] Lipworth L, Sonderman JS, Mumma MT, *et al.*, Cancer mortality among aircraft manufacturing workers: an extended follow-up, J Occup Environ Med 53(9): 992-1007 (2011).

rates were the same as the normal population.[30] TCE exposures in this cohort were directly confirmed from urinary biomonitoring of the TCE metabolite trichloroacetic acid (TCA). However, overall TCE exposures were likely low in this cohort in that most urinary TCA measurements were less than 50 mg/L, corresponding to approximately 20 ppm TCE exposure. Thus, consistent with the conclusions of Bruning *et al.* (2003),[31] this study indicates TCE is unlikely to be a low-dose kidney carcinogen.

Similarly, no evidence of kidney cancer was found by Vlaanderen *et al.* (2013) in a recent follow-up examination of the Nordic Occupational Cancer cohort (Finland, Iceland, Norway, Sweden) in which statistically non-significant risk ratios (RR) of 1.01 (0.95-1.07), 1.02 (0.97-1.08), and 1.00 (0.95-1.07) were reported for a total of 4,145 renal cancer cases approximately equally distributed across three respective TCE exposure groups (tertiles) assigned from a job exposure matrix analysis.[32] Finally, although a meta-analysis of 23 studies meeting criteria for study inclusion found a slightly increased simple summary association of TCE and kidney cancer, RR 1.42 (1.17-1.77), more detailed analyses of subgroups suggested no association, or possibly a moderate elevation in kidney cancer risk, and no evidence of increasing risk with increasing exposure.[33]

These more recent studies were not reviewed in the 2011 TCE IRIS Assessment or the 2014 TCE Work Plan Assessment that form the basis for the proposed regulation. Any regulatory action under TSCA § 6, however, is required to be based on the "best available science" supported by "substantial evidence in the record." This provides compelling support for our position that the instant proposal should be withdrawn and the uses under consideration be examined following the TCE assessment EPA will be conducting in the near future under TSCA § 6(b)(4)(A).

3. EPA's Reliance on Charbotel *et al.* (2006) Results in an Overly Conservative Estimate of Risk

In its 2014 Work Plan Assessment of potential cancer risk, EPA focused solely on inhalation exposures and relied on an inhalation unit risk (IUR) value developed in the 2011 IRIS Assessment. The IUR was based primarily on epidemiology data from the case-control study on renal cell carcinoma (RCC) by Charbotel *et al.* (2006), discussed above. Although other epidemiological studies were used to derive an adjusted IUR estimate for the combined risk of developing RCC, NHL, or liver cancer, EPA concedes a lower level of confidence in both the NHL and liver cancer databases. While the Charbotel *et al.* study suggests a relationship between cumulative TCE exposure and RCC incidence, the reliability of the exposure estimates is a major concern.

The National Academy of Sciences Committee that reviewed the draft IRIS assessment released in 2001 recommended that:

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[30] Hansen J, Sallmén M, Seldén AI, *et al.*, Risk of cancer among workers exposed to trichloroethylene: analysis of three Nordic cohort studies, J Natl Cancer Inst 105(12): 869-877 (2013).

[31] Brüning T, Pesch B, Wiesenhütter B, *et al.*, Renal cell cancer risk and occupational exposure to trichloroethylene: Results of a consecutive case-control study in Arnsberg, Germany, Am J Ind Med. 43(3): 274-285 (2003).

[32] Vlaanderen J, Straif K, Pukkala E, *et al.*, Occupational exposure to trichloroethylene and perchloroethylene and the risk of lymphoma, liver, and kidney cancer in four Nordic countries, Occup Environ Med 70(6): 393-401 (2013).

[33] Kelsh MA, Alexander DD, Mink PJ, Mandel JH. Occupational trichloroethylene exposure and kidney cancer: a meta-analysis. Epidemiology 21(1): 95-102 (2010).

> "[t]here appear to be insufficient epidemiologic data to support quantitative dose-response modeling for trichloroethylene and cancer. The committee recommends that toxicologic data be used to fit the primary dose-response model(s) and that the available epidemiologic data be used only for validation. The committee does not believe that the available information is sufficient to determine the best dose-response model for trichloroethylene."[34]

EPA should follow the recommendation of the National Academy of Sciences, which referenced the Charbotel *et al.* (2005) final study report in its review of TCE.[35] The authors' own conclusions that the study only "suggests that there is a weak association between exposures to TRI [TCE] and increased risk of RCC" argues against the existence of the robust relationship which should be required for a dose-response assessment used as the basis for regulation.[36]

The exposure assessment for the Charbotel study was based on questionnaires and expert judgment, not direct measures of exposure.[37] Worker exposure data from deceased individuals were included in the study. In contrast to living workers, who were able to respond to the questionnaires themselves, exposure information from deceased workers (22.1% of cases and 2.2% of controls) was provided by surviving family members. The authors acknowledge that "this may have led to a misclassification for exposure to TCE due to the lower levels in the quality of information collected."

Analysis of the data revealed evidence of confounding from cutting fluid exposure. Unfortunately, TCE and cutting oil were co-exposures that could not be disaggregated and the majority of

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[34] National Research Council, Assessing the human health risks of trichloroethylene: key scientific issues, National Academies Press, Washington, DC (2006); http://www.nap.edu/openbook.php?record_id=11707&page=R1.

[35] Charbotel B, Fevotte J, Hours M, *et al.*, Case-control study on renal cell cancer and occupational trichloroethylene exposure, in the Arve Valley (France), Lyon, France: Institut Universitaire de Médecine du Travail, UMRESTTE, Université Claude Bernard (2005); http://hal.archives-ouvertes.fr/docs/00/54/59/80/PDF/charbotel_octobre_05.pdf

[36] This concern was recognized by the European Chemicals Agency (ECHA) in its 2013 Chemical Safety Report on TCE: "[T]here are several concerns with this study that should be taken into consideration when assessing its use in risk assessment and hazard characterization. For example, potential selection bias, the quality of the exposure assessment, and the potential confounding due to other exposures in the work place. With respect to the potential for selection bias, no cancer registry was available for this region to identify all relevant renal cell cancer cases from the target population. Case ascertainment relied on records of local urologists and regional medical centers; therefore, selection bias may be a concern. Given the concerns of the medical community in this region regarding renal cell cancer (RCC) among screw cutting industry workers, it is likely that any cases of renal cell cancer among these workers would likely be diagnosed more accurately and earlier. It is also much more unlikely that an RCC case among these workers would be missed compared to the chance of missing an RCC case among other workers not exposed to TCE. This preference in identifying cases among screw-cutting industry workers would bias findings in an upward direction. Concerning the potential for other exposures that could have contributed to the association, screw-cutting industry workers used a variety of oils and other solvents. Charbotel *et al.* reported lower risks for TCE exposure and renal cell cancer once data were adjusted for cutting oils. In fact, they noted, 'Indeed many patients had been exposed to TCE in screw-cutting workshops, where cutting fluids are widely used, making it difficult to distinguish between cutting oil and TCE effects.' This uncertainty questions the reliability of using data from Charbotel *et al.* since one cannot be certain that the observed correlation between kidney cancer and exposure is due to trichloroethylene."

[37] Fevotte J, Charbotel B, Muller-Beauté P, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part 1: Exposure assessment, Ann Occup Hyg 50: 765-775 (2006); http://dx.doi.org/10.1093/annhyg/mel040.

the TCE exposed population, the screw cutters, could be expected to experience similar patterns of exposure for both TCE and cutting fluids (probably in aerosol form). Thus the apparent dose-response relationship for TCE could be wholly or in part the result of exposure to cutting fluids.

In their 2006 publication of the study results, the authors assigned cumulative exposures into tertiles (i.e., low, medium and high), yet the dose-response evaluation conducted as part of the IRIS Assessment relied on mean cumulative exposure levels provided at a later date.[38] Although the IRIS Assessment references the email submission of the data to EPA, it provides no detail on the technical basis for the table, raising serious transparency issues.

In an apparent acknowledgement of the uncertainty of the exposure information, Charbotel *et al.* (2006) included an evaluation of "the impact of including deceased patients (proxy interviews) and elderly patients (>80 years of age)" on the relationship between exposure to TCE and RCC. Interestingly, it was stated that "only job periods with a high level of confidence with respect to TCE exposure were considered" in the study, an apparent reference to the use of two different occupational questionnaires, one "devoted to the screw-cutting industry and a general one for other jobs." As the Adjusted Odds Ratio (OR) for the high cumulative dose group was actually higher in the censored subgroup than in the uncensored group [3.34 (1.27-8.74) vs 2.16 (1.02-4.60)], the authors cavalierly suggested that "misclassification bias may have led to an underestimation of the risk."

What the authors and EPA appear to have overlooked is that, in addressing the misclassification bias, Charbotel may also have altered the cumulative dose-response relationship. For example, in the censored subgroup there were now only 16 exposed cases (1 in the Low Group, 4 in the Medium Group and 11 in the High Group) with Adjusted ORs of 0.85, 1.03 and 3.34, respectively. If the dose-response relationship in this higher-confidence subgroup has changed, use of the lower-confidence group to calculate the IUR would have to be rigorously justified by EPA before it could be considered sufficiently robust to drive the types of decisions based on unit risk that are found in the proposed rule.

4. Use of TCE Glutathione Conjugate Derived Metabolites Dichlorovinylglutathione (DCVG) and Dichlorovinylcysteine (DCVC) in TCE Renal Toxicity and Cancer Risk Assessment Should Be Reconsidered Given Improved Understanding of the Differential Quantitative Formation of these Metabolites in Animals Relative to the TCE Oxidative Metabolites Trichloroethanol (TCOH), Trichloroacetic Acid (TCA) and Dichloroacetic Acid (DCA)

The TCE IRIS Assessment relies in part on the conclusion that DCVG and DCVC, which are weakly active renal toxicants and genotoxicants, are formed in toxicologically significant concentrations following human exposures to TCE. Importantly, the basis for this conclusion rests on studies in which a relatively high blood DCVG concentration (100 nM) was observed in volunteers exposed for 4 hours to 50 or 100 ppm TCE.[39] However, Lash *et al.* (1999) relied on a colorimetric chromatographic method analysis of TCE glutathione conjugate-derived metabolites which had substantial potential for detection of non-TCE-specific endogenous substances. Subsequent radiochemical and HPLC-MS/MS based analyses that specifically quantitated both DCVG and DCVC have found that the activity of the

[38] Charbotel B (2008) [Email from Barbara Charbotel, University of Lyon, to Cheryl Scott, EPA].

[39] Lash LH, Putt DA. Identification of S-(1,2-dichlorovinyl)glutathione in the blood of human volunteers exposed to trichloroethylene. J Toxicol Env Hlth Part A. 56: 1-21 (1999).

glutathione conjugate pathway is substantially lower than that of the oxidative pathway resulting in TCA and DCA formation in both animals and humans.[40]

Since the publication of the TCE IRIS Assessment in 2011, additional studies have evaluated the kidney concentrations of TCE oxidative and glutathione conjugate-derived metabolites in a variety of mouse strains administered 5 daily oral 600 mg/kg doses of TCE.[41] Metabolites were quantitated 2 hr after the last daily dose in that toxicokinetic evaluations had shown the approximate maximum plasma concentrations of TCA, DCA, DCVG and DCVC were observed 2 hr following oral TCE treatment.[42] Using a structure-specific HPLC-ESI-MS/MS method, Yoo *et al.* (2015) demonstrated that DCVG and DCVC were only a very small fraction of total oxidative metabolites quantitated in kidney. TCOH kidney concentrations were 2-4-fold greater than TCA, and TCA concentrations were 100-1000 greater than DCA. Importantly, DCA concentrations were 100-1000-fold greater than DCVG and DCVC, resulting in the conclusion that TCE oxidative metabolism was up to 5 orders of magnitude greater than glutathione conjugate-derived metabolism. These findings were consistent with the earlier report from Kim *et al.* (2009) in which the plasma toxicokinetics TCA, DCA, DCVG and DCVC following a single 2140 mg/kg oral TCE dose found that the cumulative AUC of oxidative metabolites was 40,000-fold higher than the combined AUC of DCVG and DCVC; note that this study did not quantify TCOH, which would have further increased the disparity of glutathione conjugate-derived relative to oxidative-derived metabolites. These data demonstrate a dramatically lower function glutathione-conjugate metabolism relative to oxidative metabolism in mice, despite the observation by Dekant (2010) that mice generate DCVC at slightly higher rates than rats and greater than 10-fold higher than humans.

The results of studies using structure-specific analytical methods for quantitation of DCVG and DCVC directly challenge the hypothesis that glutathione conjugate-derived metabolites plausibly account for the genotoxicity, renal cytotoxicity, and ultimate carcinogenicity in rodents.[43] DCVC was only marginally cytotoxic (LDH release), if at all, when incubated at 0.2M (200,000 nM) with isolated renal cortical cells of male and female rats. This *in vitro* concentration is substantially higher than the approximate maximum kidney concentrations of 10-75 nM DCVC resulting from treatment of various strains of mice with a high oral TCE dose of 600 mg/kg/day for 5 days observed by Yoo *et al.* (2015). In addition, a likely NOAEL of 1 mg/kg/day was reported for kidney toxicity (no change in serum BUN, weak tubule dilation and no necrosis) in mice administered DCVC orally or intraperitoneally at 1, 10 or 30 mg/kg/day, 1 day per week, for 13 weeks.[44] If, based on Yoo *et al.* (2015), it is assumed that the ratio of formation of oxidative metabolites to glutathione conjugate-derived metabolites is 10,000:1, an implausibly high (occupational or general population) dose of 6044 mg/kg TCE would be required to

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[40] Dekant, W (2010), attached as Appendix 4.

[41] Yoo HS, Bradford BU, Kosyk O, Uehara T, Shymonyak S, Collins LB, Bodnar WM, Ball LM, Gold A, Rusyn I. Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: kidney effects. J Toxicol Env Hlth Pt A, 78: 32-49.b (2015).

[42] Kim, S, Kim, D, Pollack, GM, Collins, LB, and Rusyn, I, Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine, Toxicol Appl Pharmacol 238: 90-99 (2009).

[43] Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Krause RJ, Parker JC, Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: Sex-, species-, and tissue-dependent differences, J Pharmacol Exp Ther 297: 155-164 (2001).

[44] Shirai N, Ohtsuji M, Hagiwara K, Tomisara H, Ohtsuje N, Hirose S, Hagiwara H, Nephrotoxic effect of subchronic exposures to S-(1,2-dichlorovinyl)-L-cysteine in mice, J Toxicol Sci 37: 871-878.h (2012).

deliver a NOAEL dose of 1 mg/kg/day DCVC (1 mmol/kg/day TCE results in 0.0001 mmol/kg/day DCVC; 1 mg/kg/day DCVC = 0.0046 mmol/kg/day). These dose-toxicity calculations suggest that it appears toxicologically implausible that real-world exposures to TCE are capable of producing doses of DCVC sufficient to cause renal toxicity and carcinogenicity in mice.

D. Peer Review Ignored

The draft Work Plan Assessment was the subject of peer review by a panel selected by EPA in 2013. The peer review report highlights that it was a screening level assessment that inappropriately relied on an unreproducible study, and recommended that the assessment be abandoned.[45] One reviewer devoted six pages to a very detailed critique of Johnson *et al.* (2003) and EPA's reliance on such a deficient study.[46] Nevertheless, EPA ignored the peer review. Remarkably, even though the trade press article on the peer review was entitled *EPA Peer Reviewers Say Trichloroethylene Analysis Not Ready for Regulatory Use*, the EPA Assistant Administrator for Chemical Safety and Pollution Prevention wrote to the EPA Inspector General that "[i]t is notable that *the external peer reviews of all the Work Plan assessments we have completed thus far supported our overall assessment methodologies and conclusions.*"[47] A more detailed description of the peer reviewers' comments is attached as Appendix 3.

Ppeer review is identified as a key step in EPA's proposed procedures for chemical risk evaluation under TSCA as amended. EPA states that "[i]n addition to any targeted peer review of specific aspects of the analysis, the entire risk assessment will also undergo peer review, as it is important for peer reviewers to consider how the various underlying analyses fit together to produce an integrated risk characterization which will form the basis of unreasonable risk determination."[48] As the draft Work Plan Assessment for TCE did not address the spot cleaning scenario at all, the assessment of risks under that scenario has *never* been subjected to peer review. Thus an applicable requirement of TSCA §§ 6 and 26(l)(4) for reliance on the Work Plan Assessment has not been met.

E. Screening Level Assessment

As noted above and in Appendix 5, the peer review report highlights that the Work Plan Assessment was a screening level assessment. Specifically, the Chairperson of EPA's peer review panel wrote:

> "The draft document fails to articulate satisfactorily that the analysis described within should be characterized as a screening level assessment. . . . I believe that the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . . After listening carefully to the comments and contributions from the other members of

[45] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

[46] *Id.*

[47] Response to Office of Inspector General Draft Report No. OPE-FY14-0012 "EPA's Risk Assessment Division Has Not Fully Adhered to Its Quality Management Plan," (July 30, 2014), Appendix A, p.10 (available at https://www.epa.gov/sites/production/files/2015-09/documents/20140910-14-p-0350.pdf) (emphasis added). *Compare* BNA Daily Environment Report, *EPA Peer Reviewers Say Trichloroethylene Analysis Not Ready for Regulatory Use* (July 18, 2013).

[48] 82 Fed. Reg. at 7572.

the Panel, I have concluded that there would little benefit in revising this draft screening assessment."

With regard to aerosol degreasing, EPA identified only two aerosol degreasing products containing TCE in the marketplace and found no emissions or monitoring data for either product – thus these are hypothetical exposures. Further, EPA used E-FAST2/CEM modeling to develop "high-end acute inhalation exposure estimates" based solely on professional judgment, providing confirmation that this is a screening level assessment. The highest uncertainties were associated with mass of product used per event, duration of event, and number of events per year, as the values selected were hypothetical, thus leading to further lack of confidence in the assessment.

For spot cleaning workers the problems with the exposure assessment are even more obvious. A major limitation of the exposure assessment used to evaluate potential risk arising from spot cleaning operations was the unavailability of relevant exposure monitoring data. Section 2.4.2.5 of the Work Plan Assessment, however, references a study specific to spot cleaning and states that "site-specific parameters from this study were incorporated into the NF/FF model to obtain site-specific model estimates of worker exposure."[49]

Examination of the NIOSH (1997) study reveals that the air monitoring was actually conducted in response to an OSHA complaint from workers and the report states that "[c]onditions at this shop were probably worst case." Use of monitoring data from a worst case, potential enforcement situation adds additional strength to the concern that the Work Plan Assessment is actually a screening level assessment which does not reflect normal operating conditions and exposures.

It is clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan Assessment that EPA carried out for TCE, which does not comply with Office of Management and Budget ("OMB") guidelines implementing the Information Quality Act.[50] First, EPA must conduct a "highly influential scientific assessment" to support TSCA § 6 rulemaking. OMB defines a scientific assessment as "highly influential" if dissemination of the assessment could have a potential impact of more than $500 million in any one year on either the public or private sector, or if the dissemination is novel, controversial, precedent-setting, or has significant interagency interest.

The TCE assessment employed worst-case or default assumptions that led to overestimation of potential risks. Such assessments may be appropriate to support a decision that no further action or evaluation is necessary, because there is confidence that the potential risks are not a concern. However, they are inappropriate to support regulations intended to reduce risk because screening level assessments do not accurately estimate risk or quantify exposures. Second, OMB's guidelines also require agencies to subject highly influential scientific assessments to more rigorous peer review. For TCE, EPA selected a contractor to manage the peer review process, even though experts consider contractor-managed peer review to be the least rigorous level of peer review.

F. Summary of Concerns

[49] National Institute for Occupational Safety and Health (NIOSH), Control of Health and Safety Hazards in Commercial Dry Cleaning, Publication Number 97-150, Centers for Disease Control and Prevention, Atlanta, GA (1997); http://www.cdc.gov/niosh/docs/97-150/#controls

[50] OMB, Final Information Quality Bulletin for Peer Review (Dec. 16, 2004) (available at https://www.whitehouse.gov/sites/default/files/omb/assets/omb/memoranda/fy2005/m05-03.pdf).

In sum, the TCE Work Plan Assessment is inconsistent with the applicable requirements of revised § 6 in the following ways, among others:

- It expressly relies on hazard values derived directly from a single academic study to estimate non-cancer risk, even though several other studies, including two GLP-compliant studies conducted under EPA guidelines, have been unable to reproduce the effect;[51]

- The University of Arizona study upon which EPA relies has been heavily criticized in the published literature,[52] and other regulatory agencies have expressly declined to rely on the academic study citing data quality concerns;[53]

- The authors of the Arizona study have published repeated corrections that fail to address the data quality concerns;[54] and a majority of EPA's own staff scientists expressed "low" confidence in its results.[55]

- The Work Plan Assessment relies on qualitative and quantitative estimates of cancer risk that are not realistic or justified by any underlying science. Two recent large Nordic epidemiological studies, both of which had extensive follow-up of the cohorts, failed to find an association between TCE and kidney cancer, but these are not addressed in the Work Plan Assessment. Further, EPA's reliance upon a potency factor based on Charbotel *et al.* (2006) directly contravenes the advice EPA received from the National Academy of Sciences

- For aerosol degreasing EPA provided no emissions or monitoring data – thus these are hypothetical exposures. The spot cleaning exposure assessment relies solely on a 2007 California study, which EPA recognized may not be representative of US dry cleaning facilities. The draft TCE Assessment, entitled "Degreaser and Arts/Crafts Uses," did not address spot cleaning at all (except to say that none of those sold to consumers contained TCE), but the final Work Plan Assessment is entitled "Degreasing, *Spot Cleaning* and Arts & Crafts Uses" and includes commercial use of TCE as a spotting agent at dry cleaning facilities.

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[51] *Compare* Johnson *et al.* (2003) to Fisher, J, *et al.*, Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development? Int. J. Toxicol. 20: 257-67 (2001) and Carney, E, *et al.*, Developmental toxicity studies in Crl:Cd (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene, Birth Defects Research (Part B) 77: 405–412 (2006).

[52] *E.g.*, "Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a 'specific' cardiac teratogen." Hardin, B, *et al.*, Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004); Watson, R., *et al.*, Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature, Repro. Toxicol. 21: 117-47 (2006).

[53] *E.g.*, "The data from this study were not used to calculate a public-health protective concentration since a meaningful or interpretable dose-response relationship was not observed. These results are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits." California EPA Public Health Goal for Trichloroethylene in Drinking Water (July 2009), at 21.

[54] Johnson, PD. *et al.*, Environ Health Perspect 122: A94 (2014): erratum to Johnson, PD, *et al.*, Environ Health Perspect 113:A18 (2005), which is an erratum to Johnson *et al.* (2003).

[55] TCE Developmental Cardiac Toxicity Assessment Update (available at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2012-0723-0045).

- It is a screening level assessment which does not meet OMB guidelines implementing the Information Quality Act for a "highly influential scientific assessment" to support TSCA § 6 rulemaking.

- The report of the peer review of the TCE Assessment highlights the foregoing points in the clearest possible terms, but EPA ignored it.[56] In fact, the EPA Assistant Administrator characterized the peer review as supportive.

Following enactment of the Lautenberg Act, it should be clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan Assessment that EPA conducted for TCE. Peer review and public comments identified numerous scientific deficiencies with the draft assessment, including the inappropriate use of default assumptions; ignoring contrary evidence that affects the weight of the scientific evidence; reliance on inapposite exposure data; conclusions inconsistent with the evidence cited; and reliance on a study that is not reproducible. Important shortcomings in both the hazard and exposure assessments were noted. Whatever "best available science" may mean, it cannot include reliance on an unreproducible toxicity study, a cancer risk assessment that does not take into account relevant epidemiological and toxicological studies, or outdated and unrepresentative exposure information.[57] And certainly EPA can no longer afford to ignore the conclusions of the peer review it initiated, as TSCA § 26(h) requires it to consider "the extent of independent verification or peer review of the information."

II. Failure to Comply with SBREFA

The Regulatory Flexibility Act, as amended by the Small Business Regulatory Enforcement Fairness Act (SBREFA), provides:

> "(a) When any rule is promulgated which will have a significant economic impact on a substantial number of small entities, the head of the agency promulgating the rule or the official of the agency with statutory responsibility for the promulgation of the rule shall assure that small entities have been given an opportunity to participate in the rulemaking for the rule through the reasonable use of techniques such as—
>
> (1) the inclusion in an advance notice of proposed rulemaking, if issued, of a statement that the proposed rule may have a significant economic effect on a substantial number of small entities;
>
> (2) the publication of general notice of proposed rulemaking in publications likely to be obtained by small entities;
>
> (3) the direct notification of interested small entities;

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[56] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

[57] *See* 162 Cong. Rec. S3522 (June 7, 2016) ("For far too long Federal agencies have manipulated science to fit predetermined political outcomes, hiding information and underlying data, rather than using open and transparent science to justify fair and objective decision making. This Act seeks to change all of that and ensure that EPA uses the best available science, bases scientific decisions on the weight of the scientific evidence rather than one or two individual cherry-picked studies, and forces a much greater level of transparency that forces EPA to show their work to Congress and the American public.)"

(4) the conduct of open conferences or public hearings concerning the rule for small entities including soliciting and receiving comments over computer networks; and

(5) the adoption or modification of agency procedural rules to reduce the cost or complexity of participation in the rulemaking by small entities.

"(b) Prior to publication of an initial regulatory flexibility analysis which a covered agency is required to conduct by this chapter—

(1) a covered agency shall notify the Chief Counsel for Advocacy of the Small Business Administration and provide the Chief Counsel with information on the potential impacts of the proposed rule on small entities and the type of small entities that might be affected;

(2) not later than 15 days after the date of receipt of the materials described in paragraph (1), the Chief Counsel shall identify individuals representative of affected small entities for the purpose of obtaining advice and recommendations from those individuals about the potential impacts of the proposed rule;

(3) the agency shall convene a review panel for such rule consisting wholly of full time Federal employees of the office within the agency responsible for carrying out the proposed rule, the Office of Information and Regulatory Affairs within the Office of Management and Budget, and the Chief Counsel;

(4) the panel shall review any material the agency has prepared in connection with this chapter, including any draft proposed rule, collect advice and recommendations of each individual small entity representative identified by the agency after consultation with the Chief Counsel, on issues related to subsections 603(b), paragraphs (3), (4) and (5) and 603(c);

(5) not later than 60 days after the date a covered agency convenes a review panel pursuant to paragraph (3), the review panel shall report on the comments of the small entity representatives and its findings as to issues related to subsections 603(b), paragraphs (3), (4) and (5) and 603(c), provided that such report shall be made public as part of the rulemaking record; and

(6) where appropriate, the agency shall modify the proposed rule, the initial regulatory flexibility analysis or the decision on whether an initial regulatory flexibility analysis is required."[58]

No Small Business Advisory Review (also referred to as "SBREFA Panel") was held for the proposed rule, however. Instead, EPA determined and certified that the rule would "not, if promulgated, have a significant economic impact on a substantial number of small entities." Where such a certification is made, no initial or final regulatory analysis is required, and thus a SBREFA Panel need not be convened.[59]

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[58] 5 U.S.C. § 609(a), (b).

[59] 5 U.S.C. § 605(b): "Sections 603 and 604 of this title shall not apply to any proposed or final rule if the head of the agency certifies that the rule will not, if promulgated, have a significant economic impact on a substantial number of small entities. If the head of the agency makes a certification under the preceding sentence, the agency

HSIA submits that EPA could not lawfully have certified that the proposed rule banning the use of TCE in spot cleaning lacked SISNOSE. EPA has adopted guidance on making the SISNOSE determination:

> "The lower economic impact threshold is particularly important because it is used to screen out rules that generally will not have a significant economic impact and, therefore, can be presumed not to require an IRFA/FRFA (i.e., if all small entities subject to a rule face economic impacts less than the lower threshold, then the rule may be assigned to the Presumed No SISNOSE Category). For this reason the lower economic impact threshold should be set conservatively, at a level that precludes any reasonable possibility that a rule placed in the Presumed No SISNOSE Category might later be found to impose a "significant economic impact on a substantial number of small entities." The upper threshold defines a level of economic impact that would be unquestionably significant for a small entity. In analyzing previous rules, EPA has often defined the lower threshold as compliance costs of 1% of sales and the higher threshold as compliance costs of 3% of sales as shown in the example in Table 2."[60]

The guidance further states that where the number of small entities subject to the rule and experiencing given economic impact is 1,000 or more, regardless of the percentage these constitute of all the small entities subject to the rule that are experiencing given economic impact, the rule will be presumed ineligible for certification.[61]

Spot cleaning is conducted by dry cleaners, virtually all of which are small businesses. The National Cleaners Association (NCA) estimates that there are some 23,550 retail dry cleaning establishments in the United States, having average sales of $250,797 and average profits of $17,809. Industry suppliers report that 60-90% of retail dry cleaners routinely order TCE for use on the spotting board (14,130 – 21,195 small businesses).

During an EO 12866 meeting on October 3, 2016, NCA provided the foregoing and following information. TCE is one of the most used spotting agents. TCE's effectiveness as a spot remover helps cleaners minimize time spent in stain removal and therefore control labor and operational costs. In most small dry cleaning plants the stain removal technician is the highest paid employee. Depending on the operation, labor represents 25-42% (average 30%) of the dry cleaners' costs. Assuming that only twelve garments a day require five additional minutes of stain removal time, this will add one hour a day to the spotter's labor. Assuming the spotter earns just $35,000 per year, one extra hour per day in a 6-day week, with overtime involved, will result in an extra $7,875 in the spotter's gross wages. It will also result in increased utilities due to six additional hours per week of boiler time and plant operation. It will also result in wasted or slowed production in the pressing department as they wait longer for cleaned

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shall publish such certification in the Federal Register at the time of publication of general notice of proposed rulemaking for the rule or at the time of publication of the final rule, along with a statement providing the factual basis for such certification."

[60] Final Guidance for EPA Rulewriters: Regulatory Flexibility Act as amended by the Small Business Regulatory Enforcement Fairness Act, https://www.epa.gov/sites/production/files/2015-06/documents/guidance-regflexact.pdf, Table 2.

[61] *Id.*

garments, further increasing labor costs. Between labor and utilities, NCA estimated *an increased cost of between 4–5% of gross sales.*[62]

Even the lowest increased cost estimated by NCA (4% of gross sales), at the low end of the range of small dry cleaning entities (14,130), constitutes SISNOSE as defined in EPA's guidance. The economic analysis in the docket acknowledges a much larger universe of dry cleaning that use spot removers (48,602) but concludes, with no factual support, that all of these are expected to experience cost impacts that are less than one percent of their revenues.[63]

Remarkably, neither the preamble to the proposed rule nor the economic analysis contains a detailed "statement providing the factual basis for such certification [of no SISNOSE] required by law." Rather, the latter includes a remarkably abstruse discussion of "market failure" that could be inserted into any analysis to support regulation in the absence of data specific to an industry or small business sector.[64] It is respectfully submitted that this does not meet the requirements of the Regulatory Flexibility Act.

III. Failure to Comply with Notice Requirements of TSCA and Administrative Procedure Act

EPA's TCE Work Plan Assessment is legally deficient in a more fundamental way. The draft Assessment was entitled "Degreaser and Arts/Crafts Uses." It states that "EPA focused the assessment on uses of TCE as a degreaser (i.e., both in small commercial settings and by consumers or hobbyists) and on consumer use of TCE in products used by individuals in the arts and crafts field" (p. 14). Spot cleaning is mentioned only in fn. 8: "there were several spot cleaners for fabrics marketed to consumers, but none contained TCE; lists of ingredients were not available for a few of the spot cleaners." There was no reference at all to spot cleaning in the workplace. Yet, with no explanation, the final TCE Work Plan Assessment is entitled "Degreasing, *Spot Cleaning* and Arts & Crafts Uses" and includes "Commercial use of TCE as a spotting agent at dry cleaning facilities" (p. 26).

The failure to notify dry cleaners that EPA was assessing a key agent upon which they rely clearly violates TSCA § 6(b)(4)(H), which states: "The Administrator shall provide no less than 30 days

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[62] https://www.reginfo.gov/public/do/viewEO12866Meeting?viewRule=true&rin=2070-AK03&meetingId=2352&acronym=2070-EPA/OCSPP

[63] *See* Economic Analysis of Proposed TSCA Section 6 Action on Trichloroethylene in Dry Cleaning Spot Removers and Aerosol Degreasers, at ES-15. The difference in number of establishments is due to EPA's reliance on data from decades ago when dry cleaning was a much larger sector.

[64] It begins:

> "Market failure can justify government regulation; the major types of market failures include the following:
>
> - Negative externalities, common property resources, and public goods;
> - Market power;
> - Inadequate or asymmetric information.
>
> The occurrence of any of these conditions justifies further inquiry into the need for government regulation to reduce inefficiencies in the allocation of society's resources. This section describes why negative externalities and inadequate or asymmetric information are present in the market for dry cleaning spot removers and aerosol degreasing products."

Id. at 2-2.

public notice and an opportunity for comment on a draft risk evaluation prior to publishing a final risk evaluation." That this is an "applicable requirement[] of § 6" for purposes of TSCA § 26(l)(4), which sets forth the requirements for EPA to rely upon risk assessments completed prior to enactment of the Lautenberg Act, should be obvious. In addition, § 553 of the Administrative Procedure Act (APA) requires all federal agencies to provide public notice and an opportunity for comment on all proposed rules.[65] The APA definition of "rule" is broad and encompasses background data upon which the rule is based.

Because there was no notice that EPA was addressing spot cleaning, there was no participation by dry cleaner representatives and no peer review of the spot cleaning assessment. EPA based estimates of workers/bystanders on census data "not adjusted to exclude job categories that likely would not be present at dry cleaning facilities. Thus, EPA's estimate likely overestimates the size of the population exposed."[66] Moreover, EPA relied solely on a 2007 California study, which it recognized may not be representative of US dry cleaning facilities. As dry cleaners had no notice that EPA was assessing spot cleaning in the workplace, they did not have an opportunity to comment on the exposure estimates or the study. Thus, the minimal requirements of administrative procedure have not been met in this rulemaking.

An equally serious notice issue is presented by EPA's acknowledgement that it only evaluated the commercial use of TCE for spot cleaning at dry cleaning facilities in the final Work Plan Assessment in response to a peer reviewer comment. It is therefore obvious that the evaluation of this additional use in the final risk assessment was not itself actually peer reviewed. Similarly, the supplemental analyses conducted by EPA to identify risks for the commercial aerosol degreasing use scenario and for various parameters of exposure scenarios for TCE spot cleaner use in dry cleaning facilities were only done long after completion of the Work Plan Assessment and after passage of the Lautenberg Act. Further, these analyses have not been peer reviewed. As noted above, peer review of these analyses is required by the OMB Final Information Quality Bulletin for Peer Review and TSCA.

IV. EPA's Reliance on Alternatives is Unrealistic

TSCA § 6(c)(2) provides:

> "(C) CONSIDERATION OF ALTERNATIVES.—
>
> "Based on the information published under subparagraph (A), in deciding whether to prohibit or restrict in a manner that substantially prevents a specific condition of use of a chemical substance or mixture, and in setting an appropriate transition period for such action, the Administrator shall consider, to the extent practicable, whether technically and economically feasible alternatives that benefit health or the environment, compared to the use so proposed to be prohibited or restricted, will be reasonably available as a substitute when the proposed prohibition or other restriction takes effect."

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[65] 5 U.S.C. § 553(b), (c): "General notice of proposed rulemaking shall be published in the Federal Register, unless persons subject thereto are named and either personally served or otherwise have actual notice thereof in accordance with law. . . . After notice required by this section, the agency shall give interested persons an opportunity to participate in the rulemaking through submission of written data, views, or arguments with or without opportunity for oral presentation."

[66] TCE Work Plan Assessment, at 116.

The proposal suggests that n-propyl bromide (nPB), perchloroethylene, methylene chloride, and water-based compounds could be used as alternatives to TCE in spot cleaning. Many of these alternatives are ineffective, hence the continued market dominance of the TCE-based products. Moreover, there is serious question whether a number of these alternatives would realistically be available, given the designation of nPB, perchloroethylene, and methylene chloride as priorities for risk evaluation/regulation under TSCA § 6(b)(2)(A).[67]

Query how compounds such as nPB could be considered a "reasonably available" substitute for TCE, much less how EPA could consider making such a finding in light of the fact that substitution on nPB in foam fabrication following reduction of the workplace limit for methylene chloride is regarded as a textbook example of "regrettable substitution." Unlike TCE, which has a long history of safe use in the workplace, the serious health impairments suffered by workers in those facilities have been widely documented. Moreover, an nPB industry representative stated at EPA's February 14, 2017 meeting on scoping documents for the ten priority compounds that nPB is no longer used in dry cleaning at all.

V. Gap Filling Purpose of TSCA

As originally enacted and as updated by the Lautenberg Act, TSCA requires EPA to consult and coordinate with other federal agencies "for the purpose of achieving the maximum enforcement of this Act while imposing the least burdens of duplicative requirements on those subject to the Act and for other purposes."[68] Worker and consumer health and safety fall under the jurisdictions, respectively, of OSHA and the Consumer Product Safety Commission (CPSC). The use of TCE in spot cleaning and aerosol degreasing is already more than adequately regulated under the OSH Act and/or the Federal Hazardous Substances Act. This comprehensive regulatory framework provides adequate protections with respect to the same potential adverse impacts and potential exposure pathways targeted by the proposed rule. Taking steps that may lead to the removal of products from the marketplace because workers or consumers failed to comply with the existing legal requirements is not consistent with TSCA either as initially enacted or as revised.

The basis for EPA's broad assertion of jurisdiction over occupational and consumer uses is unclear. The Lautenberg Act eliminated the requirement in TSCA § 6(a) that EPA protect "against [unreasonable] risk using the least burdensome requirements," but did not materially change the existing framework that requires unreasonable risks to be addressed under statutory authority other than TSCA wherever possible. EPA's longstanding interpretation of this framework is as follows:

> "Under section 9(a)(1) of TSCA, the Administrator is required to submit a report to another Federal agency when two determinations are made. The first determination is that the Administrator has reasonable basis to conclude that a chemical substance or mixture presents or will present an unreasonable risk of injury to health or the environment. The second determination is that the unreasonable risk may be prevented or reduced to a sufficient extent by action taken by another Federal agency under a Federal law not administered by EPA. Section 9(a)(1) provides that where the Administrator makes these two determinations, EPA must provide an opportunity to the other Federal agency to assess the risk described in the report, to interpret its own statutory authorities, and to initiate an action under the Federal laws that it administers.
>
> "Accordingly, section 9(a)(1) requires a report requesting the other agency: (1) To determine if the risk may be prevented or reduced to a sufficient extent by action taken

[67] 81 Fed. Reg. 91927 (Dec. 19, 2016).

[68] TSCA § 9(d).

under its authority, and (2) if so, to issue an order declaring whether or not the activities described in the report present the risk described in the report.

"Under section 9(a)(2), EPA is prohibited from taking any action under section 6 or 7 with respect to the risk reported to another Federal agency pending a response to the report from the other Federal agency. There would be no similar restriction on EPA for any risks associated with a chemical substance or mixture that is not within the section 9(a)(1) determinations and therefore not part of the report submitted by EPA to the other Federal agency."[69]

It was clear from the outset that TSCA is to be used only when other statutes fail to provide a remedy for unreasonable risks. When TSCA was enacted in 1976, Representative James Broyhill of North Carolina indicated that "it was the intent of the conferees that the Toxic Substance Act not be used, when another Act is sufficient to regulate a particular risk."[70] TSCA § 9(a) is substantively unchanged by the Lautenberg Act. The House Energy and Commerce Committee Report states: "H.R. 2576 reinforces TSCA's original purpose of filling gaps in Federal law that otherwise did not protect against the unreasonable risks presented by chemicals," and further clarifies that "while § 5 makes no amendment to TSCA § 9(a), the Committee believes that the Administrator should respect the experience of, and defer to other agencies that have relevant responsibility such as the Department of Labor in cases involving occupational safety."[71]

Colloquies on the floor of the House of Representatives make this intent clear with specific reference to TCE, most notably the following:

"Mr. SHIMKUS. Mr. Speaker, I yield 2 minutes to the gentlewoman from Tennessee (Mrs. *Blackburn*), the vice chair of the full committee.

Mrs. BLACKBURN. Mr. Speaker, I do rise in support of the amendments to H.R. 2576, and I congratulate Chairman *Shimkus* on the wonderful job he has done. Mr. Speaker, I yield to the gentleman from Illinois (Mr. *Shimkus*) for the purpose of a brief colloquy to clarify one important element of the legislation.

Mr. Chairman, it is my understanding that this bill reemphasizes Congress' intent to avoid duplicative regulation through the TSCA law. It does so by carrying over two important EPA constraints in section 9 of the existing law while adding a new, important provision that would be found as new section, 9(b)(2).

It is my understanding that, as a unified whole, this language, old and new, limits the EPA's ability to promulgate a rule under section 6 of TSCA to restrict or eliminate the use of a chemical when the Agency either already regulates that chemical through a different statute under its own control and that authority sufficiently protects against a risk of injury to human health or the environment, or a different agency already regulates that chemical in a manner that also sufficiently protects against the risk identified by EPA.

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[69] 4,4'-Methylenedianiline; Decision to Report to the Occupational Safety and Health Administration, 50 Fed. Reg. 27674 (July 5, 1985). EPA also has acted under § 9(a) to refer 1,3-butadiene and glycol ethers to OSHA, 50 Fed. Reg. 41393 (Oct. 10, 1985) and 51 Fed. Reg. 18488 (May 20, 1986), respectively, and to refer dioxins in bleached wood pulp and paper products to the Food and Drug Administration, 55 Fed. Reg. 53047 (Dec. 26, 1990).

[70] 122 Cong. Rec. H11344 (Sept. 28, 1976).

[71] H. Rep. No. 114-176 (114th Cong., 1st Sess.) at 28.

Would the chairman please confirm my understanding of section 9?

Mr. SHIMKUS. Will the gentlewoman yield?

Mrs. BLACKBURN. I yield to the gentleman from Illinois.

Mr. SHIMKUS. The gentlewoman is correct in her understanding.

Mrs. BLACKBURN. I thank the chairman. The changes you have worked hard to preserve in this negotiated bill are important. As the EPA's early-stage efforts to regulate methylene chloride and TCE under TSCA statute section 6 illustrate, they are also timely.

EPA simply has to account for why a new regulation for methylene chloride and TCE under TSCA is necessary since its own existing regulatory framework already appropriately addresses risk to human health. New section 9(b)(2) will force the Agency to do just that.

I thank the chairman for his good work."[72]

Indeed, TSCA § 9 was strengthened by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, and it was clear from the outset that TSCA is to be used only when other statutes fail to provide a remedy for unreasonable risks. Representative James Broyhill of North Carolina indicated that "it was the intent of the conferees that the Toxic Substance Act not be used, when another act is sufficient to regulate a particular risk."[73] EPA applied this statutory directive in determining that the risk from 4,4' methylenedianiline (MDA) could be prevented or reduced to a significant extent under the Occupational Safety and Health Act, and referring the matter for action by OSHA.[74] And in an analysis of TSCA § 9, EPA's Acting General Counsel concluded that "Congress expected EPA – particularly where the Occupational Safety and Health Act was concerned – to err on the side of making referrals rather than withholding them."[75]

There is no evidence that EPA has submitted to OSHA "a report which describes such risk and includes in such description a specification of the activity or combination of activities which the Administrator has reason to believe so presents such risk and includes in such description a specification of the activity or combination of activities which the Administrator has reason to believe so presents such risk," as required by TSCA § 9(a)(1). The non-existent report obviously did not "include a detailed statement of the information on which it is based" and was not "published in the Federal Register," as required.

Had the required report been issued, it presumably would have identified how OSHA's authority over the workplace was insufficient to address the risks posed by spot cleaning and aerosol degreasing using TCE. A letter from the Assistant Secretary of Labor for Occupational Safety and Health (undated but apparently issued on April 4, 2016) identifies limits on OSHA's authority to regulate hazardous substances such as TCE, but it does not come close to meeting the requirements of TSCA for EPA action in this case. The April 2016 letter identifies no gap specific to spot cleaning or aerosol degreasing in any particular category of workplace, rather it simply recites how OSHA's authority does not extend to self-employed

[72] 162 Cong. Rec. H3028 (May 24, 2016).

[73] 122 Cong. Rec. H11344 (Sept. 28, 1976).

[74] 50 Fed. Reg. 27674 (July 5, 1985).

[75] Memorandum to Lee M. Thomas from Gerald H. Yamada, June 7, 1985, p. 2.

workers, military personnel, and consumer uses. But those are limitations that were imposed by Congress and have existed since the Occupational Safety and Health Act was enacted (six years before enactment of TSCA). Those limitations apply to every use of every toxic substance. Congress cannot have meant, in enacting "gap-filling" legislation, to open the door to EPA assuming all authority over the use of hazardous substances in the workplace.

If EPA were to identify a category of exposure deemed to present a risk that is unreasonable, these considerations indicate that referral under § 9(a) would be the appropriate course.[76] It is clear from Section 9(a) that TSCA is to be used only when other statutes fail to provide a remedy for unreasonable risks.

Attachments:

Appendix 1
Appendix 2
Appendix 3
Appendix 4
Appendix 5

[76] As noted above, TSCA § 9(a) provides that if the Administrator has reasonable basis to conclude that an unreasonable risk of injury is presented, and he determines, in his discretion, that the risk may be prevented or sufficiently reduced by action under another federal statute not administered by EPA, then the Administrator shall submit a report to that agency describing the risk. In the report, the Administrator shall request that the agency determine if the risk can be prevented or sufficiently reduced by action under the law administered by that agency; if so, the other agency is to issue an order declaring whether the risk described in the Administrator's report is presented, and is to respond to the Administrator regarding its prevention or reduction. The Administrator may set a time (of not less than 90 days) within which the response is to be made. The other agency must publish its response in the Federal Register. If the other agency decides that the risk described is not presented, or within 90 days of publication in the Federal Register initiates action to protect against the risk, EPA may not take any action under § 6 of TSCA.

APPENDIX 2

Comments on the Weight of Evidence Cancer Conclusions in the Trichloroethylene: Consideration of Both Toxicological and Epidemiologic Evidence - External Review Draft

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Summary

These comments address the question of whether the overall toxicological and epidemiologic data provide sufficient evidence for description of TCE as "Carcinogenic to Humans." First we review the Environmental Protection Agency's (EPA's) 2005 guidelines for weight of evidence descriptors regarding carcinogenic potential . We then consider where the scientific evidence from toxicological and epidemiologic research best fits under these criteria.

Our key overall observations and conclusions are as follows: EPA has proposed a cancer descriptor of "carcinogenic to humans" for TCE "based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer."

Upon a critical scientific assessment, we find that the currently available are clearly not convincing of a causal association between TCE exposure and cancer in humans. This is because neither the epidemiologic data nor the animal and mechanistic data meet EPA's criteria of "carcinogenic to humans" as described in the 2005 EPA Guidelines for Carcinogen Risk Assessment. Moreover, we find that EPA has not judged any other chemical as a "human carcinogen" or its equivalent (using older guidelines) on such inconsistent support and such a lack of strong and convincing epidemiologic evidence. EPA's proposal to use the classification

"carcinogenic to humans" for TCE would be a poorly supported precedent in the application of its own guidelines.

Rather, our judgment based on the 2005 EPA Guidelines for Carcinogen Risk Assessment, which EPA has established to make such determinations consistent across chemical assessments, indicates that a more correct classification for EPA to make for TCE would either be "likely to be carcinogenic to humans" or "suggestive evidence of carcinogenicity" depending on how one considers the "adequacy" of evidence to demonstrate carcinogenic potential.

Summary of EPA Guidelines

The EPA's (2005) Guidelines for Carcinogen Risk Assessment suggest the following descriptors as an introduction to the weight of evidence (WOE) narrative, noting that the entire narrative provides the conclusions and the basis for them:

- Carcinogenic to humans,
- Likely to be carcinogenic to humans,
- Suggestive evidence of carcinogenicity,
- Inadequate information to assess carcinogenic potential, and
- Not likely to be carcinogenic to humans.

According to the guidelines, the descriptor "**carcinogenic to humans**" "indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- "This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

- Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* [italics added] of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action (MOA) but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on MOA and also an indication of the relative weight that each source of information carries, e.g., based on human information, based on limited human and extensive animal experiments."

According to the guidelines, the descriptor "**likely to be carcinogenic to humans**" is "appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor 'Carcinogenic to Humans.' Adequate evidence consistent with this descriptor covers a broad spectrum.... Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer;
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site or exposure route, with or without evidence of carcinogenicity in humans;

- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy or an early age at onset;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence."

According to the guidelines, the descriptor "**suggestive evidence of carcinogenicity**" is "appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples [of supporting data for this descriptor] include:

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans;"
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;

- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence; or
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

According to the guidelines, the descriptor "**inadequate information to assess carcinogenic potential**" is "appropriate when available data are judged inadequate for applying one of the other descriptors. Additional studies generally would be expected to provide further insights. Some examples include:

- little or no pertinent information;
- conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative;
- negative results that are not sufficiently robust for the descriptor, "not likely to be carcinogenic to humans."

Application of the Guidelines to Trichloroethylene

In considering the data in the context of applying the "carcinogenic to humans" descriptor, one first considers the weight of the epidemiological evidence. We judge the epidemiologic evidence to be neither "convincing" nor "strong," two key terms in the guidelines. This judgment is based on four recent reviews and meta-analyses of occupational TCE exposures and cancer as well as other reviews of this literature (Alexander et al., 2006, 2007; Mandel et al., 2006; Kelsh et al., 2010). The recent review and meta-analysis by Kelsh et al., 2010 focuses on occupational TCE exposure and kidney cancer, and includes the recent Charbotel 2006 study that is emphasized in the EPA assessment and used by EPA scientists to conduct a quantitative risk

assessment. Both the EPA meta-analysis and the recently published Kelsh et al. meta-analysis of the TCE-kidney cancer epidemiologic literature produced similar summary results. However in Kelsh et al., the limitations of this body of research, namely exposure assessment limitations, potential unmeasured confounding, potential selection biases, and inconsistent findings across groups of studies, did not allow for a conclusion that there is sufficient evidence of a casual association, despite a modest overall association. In addition, although the recent Charbotel et al. 2006 study has made important improvements in exposure assessment, it still has important potential limitations that do not permit an appropriate use in quantitative risk assessment.

There are reasonably well designed and well conducted epidemiologic studies that report no association between TCE and cancer, some reasonably well designed and conducted studies that did report associations between TCE and cancer, and finally some relatively poorly designed studies reporting both positive and negative findings. Overall, the summary relative risks or odds ratios in the meta-analysis studies (EPA or published meta-analyses) generally ranged between 1.2 and 1.4. The IRIS document refers to these associations as "small," a term not typically consistent with "convincing" and strong." Weak or small associations may be more likely to be influenced or be the result of confounding or bias. Smoking and body mass index are well-established risk factors for kidney cancer, and smoking and alcohol are risk factors for liver cancer, yet the potential impact of these factors on the meta-analysis associations was not fully considered. There were suggestions that these factors may have impacted findings (e.g. in the large Danish cohort study of TCE exposed workers, the researchers noted that smoking was more prevalent among the TCE exposed populations however little empirical data were provided (Raaschou-Nielson et al., 2003). In addition, colinearity of occupational exposures (i.e., TCE exposure correlated with chemical and/or other exposures) may make it difficult to isolate

potential effects of TCE from those of other exposures within a given study, and hinder interpretation across studies. For example, although Charbotel et al. (2006) reported potential exposure response trends, while controlling for many confounders of concern (which strengthens the weight of evidence), they also reported attenuated associations for cumulative TCE exposure *after adjustment for exposure to cutting fluids and other petroleum oils (weakening the weight of* the evidence). This study is also be limited due to other by potential study design considerations such as selection bias, self report of work histories, residual confounding and other design factors.

When examining the data for TCE and non-Hodgkin lymphoma, kidney cancer, and liver cancer, associations were inconsistent across occupational groups (summary results differed between aerospace/aircraft worker cohorts compared with workers from other industries), study design, location of the study, quality of exposure assessment (e.g., evaluating studies that relied upon biomonitoring to estimate exposure vs. semi-quantitative estimates vs. self-report, etc.), and by incidence vs. mortality endpoints. Although EPA examined high dose categories, it did not evaluate any potential dose-response relationships across the epidemiologic studies (except for the Charbotel et al. 2006 study). In our reviews of the epidemiologic data reported in various studies for different exposure levels (e.g. cumulative exposure and duration of exposure metrics), we did not find consistent dose-response associations between TCE and the three cancer sites under review (Mandel et al., 2006; Alexander et al., 2007; Kelsh et al., 2010) An established dose-response trend is one of the more important factors when making assessments of causation in epidemiologic literature. These issues are addressed in greater detail in the accompanying comments by Michael Kelsh and Dominic Alexander.

Thus, based on an overall WOE analysis of the epidemiologic research, these data do not support the conclusion that there is "strong" or "convincing" evidence of a causal association between human exposure and cancer.

The EPA's 2005 guidelines also state that a chemical may be described as carcinogenic to humans with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence, all of which must be met. One of these lines of evidence is "extensive evidence of carcinogenicity in animals." Therefore, we now turn to an evaluation of the animal data.

In weighing the evidence in experimental animals and addressing the impact of the metabolites produced, EPA states that

> "A greater variability of response is expected than from exposure to a single agent making it particularly important to look at the TCE database in a holistic fashion rather than the results of a single study, especially for quantitative inferences." (EPA, page 4-233)

We agree with EPA that the database needs to be viewed holistically. EPA goes on to surmise that evidence for cancer is found in two species (rats and mice) and for more than one tumor endpoint (kidney, liver, lung and immune system). However, EPA's description of this evidence is unconvincing when starting from the neutral question of: "Does TCE cause cancer in experimental animals?" Of the 4 primary tissues that EPA evaluates for carcinogenicity, only one or perhaps two of them, liver and lung tumors in mice, rises to the level of biological significance. Discussion of the remaining tumor types appears to presuppose that TCE is carcinogenic. The resulting text appears then to overly discount negative data, of which there are many, and to highlight marginal findings. The text does not appear to be a dispassionate rendering of the available data.[1]

[1] For example, EPA (page 4-261) states "For rats, Maltoni et al. (1986) reported 4 liver angiosarcomas (1 in a control male rat, 1 both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and 1 in a

Specifically, EPA's conclusion that kidney cancer is evident in rats rests on one statistically significant finding in over 70 dose/tumor endpoint comparisons and references to exceedances of historical control values (NTP, 1990). Using a 0.05 p-value for statistical significance, a frequency of 1 or even several statistically or biologically significant events is expected in such a large number of dosed/tumor groups. This expectation is met, but not exceeded, as shown in Tables 1 and 2, which present the percent response for the various studies of kidney tumors, grouped by exposure level. EPA notes several other occurrences of kidney tumors, but the incidence was either not statistically significant or of borderline significance in comparison with concurrent controls. The presentation of data vs. the historical NTP controls is very useful. But historical control data needs to be presented in the context of both the study and year, since drift occurs in animal colonies (e.g., it is likely that the historical control data were different for the NCI 1976 study than for the NTP 1988-1990 studies). At least as importantly, historical control data is needed for each strain, particularly in light of the relatively high control response (7% in the inhalation study in Han:Wistar rats (Henschler et al., 1980). The statements about consistent increases of a rare tumor seem to assume that the background for all strains is the same as that reported by NTP for F344 rats. Moreover, each of the studies EPA cites has

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female rat exposed to 600-ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular "hepatomas" in treated and control rats were not given. Although Maltoni et al. (1986) concluded that the small number was not treatment related, ***the findings were brought forward*** [emphasis added] because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials." Perhaps we missed them in EPA's tome, but these data were not shown.

Another example of this tendency to discount negative findings is found on Page 4-263. "Although the mice in the two experiments [Maltoni et al., 1988, Table 4-55, page 4-258] in males were of the same strain, the background level of liver cancer was significantly different between mice from the different sources (1/90 versus 19/90), though the early mortality may have led to some censoring." Perhaps we missed EPA's point, but it appears that the Table 4-55 only presented one of the two control groups. Inclusion of the control group with the higher background level would suggest that there was no chemical-related increase.

problems. Although EPA generally does a good job of identifying these problems, its overall conclusion, based on these flawed studies cannot be that TCE is a known kidney tumorigen. The best that can be said is that the data are inconsistent.

EPA states that liver tumors are statistically significant in mice. This statement is confirmed by a biological judgment of all available data as shown in Tables 5 and 6.[2]

EPA finds three statistically significant occurrences of lung tumors in mice, 1 of them in a study with known epichlorohydrin contamination. Findings in other studies might be considered as biologically significant (see highlights in Tables 9 and 10 of these comments). The rest of the studies show no statistically significant increase, or show no lung tumors, or show a decrease in lung tumors as shown in Tables 7, 8, 9 and 10. Briefly, these data are either equivocal or marginally positive. EPA might consider revising its lung tumor table (Table 4-73) in order to make this information more readily transparent.

EPA states on page 4-397 that:

> "Cancers of the immune system that have been observed in animal studies and are associated with TCE exposure are summarized in Tables 4-68 and 4-69. The specific tumor types observed are malignant lymphomas, lymphosarcomas, and reticulum cell sarcomas in mice and leukemias in rats…

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[2] EPA (page 4-261) also states that "The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F1 mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related non-neoplastic liver lesions in males and a decrease in basophilic cytological change reported from TCE-exposure in female rats. **The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error [emphasis added].**

Note well, however, that NTP (1990) is the same study in which the ***sole*** statistically significant finding of kidney cancer in rats was made by EPA (page 4-179, Table 4-41). Thus, EPA appears to accept the findings of NTP (1990) when the result is positive (kidney), but not when the result is negative (liver).

EPA then continues on page 4-399 with:

> "In summary, overall there is limited available data in animals on the role of TCE in lymphomas and leukemias. There are few studies that analyze for lymphomas and/or leukemias. Lymphomas were described in four studies (NTP, 1990; NCI, 1976; Henschler et al., 1980, 1984) but study limitations (high background rate) in most studies make it difficult to determine if these are TCE-induced. Three studies found positive trends in leukemia in specific strains and/or gender (Maltoni et al., 1986, 1988; NTP, 1988). Due to study limitations, these trends cannot be determined to be TCE-induced."

In reading the text between these two apparently disparate quotes, the data for these cancers is overwhelmingly negative; some data might be statistically significant negative (Henchler et al., 1984). The use of EPA (2005) would suggest that these experimental animals findings are negative.

As currently written, the best argument that EPA can make with these experimental animal data is that the data provide **suggestive evidence of carcinogenicity**. A holistic viewpoint, one that EPA espouses, limits the interpretation and reliability of the animal data, and/or decreases the weight of evidence for carcinogenicity in rodents. Based on these considerations, the animal data for these four tumors do not meet the criterion of "extensive evidence of carcinogenicity in animals." Multiple marginal findings do not constitute "extensive evidence." We encourage EPA to either revise its text, with appropriate supporting data, to support a judgment of "likely to cause cancer in humans," or reconsider its conclusion based on these experimental animal data.

The epidemiologic literature on TCE can be characterized by many of the terms used to describe characteristics of the "suggestive" descriptor. These include the findings of a small increase in risk of tumors (kidney, NHL, liver) combined with the possibility that these cancers can be attributable to other known and unknown factors, and where there are studies that report positive responses, the limitations in study power, design, or conduct limit the ability to draw

"confident" conclusions. As shown in the data extracted from IRIS and presented in Table 11, the epidemiological data supporting a conclusion of "known" human carcinogen, or "A carcinogen" for other chemicals under the 1986 guidelines, is typically much stronger than the data for TCE.

The available experimental animal evidence can be interpreted in various ways depending on how EPA chooses to revise its text. As currently written, this evidence is primarily negative or conflicting for kidney and immune tumors, and positive for mouse liver tumors and lung tumors, and thus the overall weight of evidence considering both epidemiology and experimental animal evidence would be best seen as "suggestive." However, a more complete presentation and analysis of the animal data may push the overall classification into the "likely" category based on a "suggestive" characterization of the epidemiologic literature and consideration of the weight of evidence from the animal tumor data, particularly the data on liver tumors in mice.

However, in no circumstance is it scientifically reasonable to judge that TCE is "carcinogenic to humans" based on the available human and experimental animal data.

In summary, a review of the available epidemiologic evidence and related meta-analyses, and the experimental animal data as presented in the document indicate "**suggestive evidence of carcinogenic potential**" of TCE based on the EPA cancer guidelines. The overall database may indicate that TCE is at the low end of "likely human carcinogen," but the document as written does not currently make that case. Description of TCE as a known human carcinogen is precluded by:

- Methodological and analytical inconsistencies in the epidemiologic literature, such as weak summary associations, differences in results by sub-groups, lack of evidence of

dose-response relationships or insufficient data to fully evaluate exposure trends, and the potential influence of confounding by lifestyle or occupational factors.

Description of TCE as a likely carcinogen based on the draft EPA text is:

- Downweighted by the conflicting or negative experimental animal data for kidney and immune tumors, and weakly supported by the positive findings for mouse liver and lung tumors.
- EPA could improve its determination of kidney tumors findings by conducting a complete historical control analysis for each study that it deems scientifically credible, but it will need to re-evaluate NTP 1990 to determine whether this study meets these criteria. EPA should not discount the negative findings for NTP (1990) for rat liver tumors, but then accept the same study for findings of rat kidney tumors.[2]

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Table 11. Summary of the Number of Positive and Negative Studies for "Known" or "A" Human Carcinogens

Chemical (Year of Assessment)	Epi Positive[1]	Epi Negative[2]	Animal Positive	Animal Negative	Rare[3]
Arsenic, inorganic (1994)	14	ND	1	4	N
Asbestos (1987)	7(9)	1	3	3	Y (mesothelioma)
Benzene (1998)	5(11)[4]	ND	-[4]	-	N
Benzene (2000 oral)	-[4]	-	4(9)	ND	N
Benzene (1998 inhalation)	-	-	3(5)	ND	N
Benzidine (1986)	5	ND	1	4	N
Bis (chloromethyl) ether (BCME) (1988)	6	ND	1	4	N
Chloromethyl methyl ether (CMME) (1987)	9	ND	3	4	N
Chromium (VI) (1998)	25(30)	ND	5	8	Y
Coke oven emissions (1989)	6(8)	2	2	2	Y
Nickel Refinery Dust (1987)	6	ND	1	9	N
Nickel subsulfide (1987)	5	1	2	4	Y

Vinyl Chloride (2000)	11(16)	2	8(10)	6(8)	Y (angio-sarcoma)
1,3-Butadiene (2001)	7(9)	ND	1	1	N

[1] First number is the best estimate of number of unique cohorts, based on the IRIS summary. The number in parentheses is total number of citations of studies.

[2] ND = not determinable from writeup; no studies were mentioned, but it is not clear from the writeup whether negative studies exist, but were not included because a strength of evidence approach was in use at the time.

[3] Tumor associated with the chemical exposure has a very low background in humans, increasing the specificity of the association.

[4] There is one IRIS assessment for benzene, with portions from 1998 and 2000. The human data are presented in the initial 1998 assessment, while inhalation data for animals were presented in the 1998 document, and oral animal data presented in a 2000 document.

APPENDIX 3

Contaminated Water Supplies at Camp Lejeune, Assessing Potential Health Effects National Research Council of the National Academy of Sciences (2009)

BOX 1 Five Categories Used by IOM to Classify Associations

Sufficient Evidence of a Causal Relationship

Evidence from available studies is sufficient to conclude that a causal relationship exists between exposure to a specific agent and a specific health outcome in humans, and the evidence is supported by experimental data. The evidence fulfills the guidelines for sufficient evidence of an association (below) and satisfies several of the guidelines used to assess causality: strength of association, dose-response relationship, consistency of association, biologic plausibility, and a temporal relationship. . . .

Sufficient Evidence of an Association

Evidence from available studies is sufficient to conclude that there is a positive association. A consistent positive association has been observed between exposure to a specific agent and a specific health outcome in human studies in which chance and bias, including confounding, could be ruled out with reasonable confidence. For example, several high-quality studies report consistent positive associations, and the studies are sufficiently free of bias, including adequate control for confounding.

Limited/Suggestive Evidence of an Association

Evidence from available studies suggests an association between exposure to a specific agent and a specific health outcome in human studies, but the body of evidence is limited. . . .

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

Evidence from available studies is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

Limited/Suggestive Evidence of No Association

Evidence from well-conducted studies is consistent in not showing a positive association between exposure to a specific agent and a specific health outcome after exposure of any magnitude. . . .

Source: IOM (Institute of Medicine). 2003. Gulf War and Health, Vol. 2, Insecticides and Solvents. Washington, DC: National Academies Press.

Contaminated Water Supplies at Camp Lejeune, Assessing Potential Health Effects National Research Council of the National Academy of Sciences (2009)

BOX 2 Categorization of Health Outcomes[a] Reviewed in Relation to TCE, PCE, or Solvent Mixtures

Sufficient Evidence of a Causal Relationship

- No outcomes

Sufficient Evidence of an Association

- No outcomes

Limited/Suggestive Evidence of an Association

- Kidney cancer
- Adult leukemia (solvent mixtures)
- Multiple myeloma (solvent mixtures)
- Myelodysplasic syndromes (solvent mixtures)
- Scleroderma (solvent mixtures)
- Neurobehavioral effects (solvent mixtures)

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

- Oral/pharyngeal cancer
- Nasal cancer
- Laryngeal cancer
- Esophageal cancer (TCE)
- Stomach cancer
- Colon cancer
- Rectal cancer
- Pancreatic cancer
- Hepatobiliary cancer
- Lung cancer (TCE)
- Bone cancer
- Soft tissue sarcoma
- Melanoma
- Non-melanoma skin cancer
- Breast cancer (TCE)
- Cervical cancer
- Ovarian/uterine cancer
- Prostate cancer
- Bladder cancer (TCE)
- Cancer of the brain or central nervous system
- Non-Hodgkin lymphoma
- Hodgkin disease
- Multiple myeloma
- Adult leukemia
- Myelodysplasic syndromes
- Childhood leukemia
- Childhood neuroblastoma
- Childhood brain cancer
- Aplastic anemia
- Congenital malformations
- Male infertility
- Female infertility (after exposure cessation)
- Miscarriage, preterm birth, or fetal growth restriction (from maternal preconception exposure or paternal exposure)
- Preterm birth or fetal growth restriction (from exposure during pregnancy)
- Cardiovascular effects
- Liver function or risk of cirrhosis
- Gastrointestinal effects
- Renal toxicity
- Amyotrophic lateral sclerosis
- Parkinson disease
- Multiple sclerosis
- Alzheimer disease
- Long-term reduction in odor discrimination
- Long-term hearing loss
- Long-term reduction in olfactory function

Limited/Suggestive Evidence of No Association

- No outcomes

[a]*Outcomes for TCE and PCE unless otherwise specified**

* PCE-only outcomes omitted

APPENDIX 4

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Würzburg, 20.01.2010

I have been asked to comment on the IRIS Document on trichloroethylene (TCE) by the Halogenated Solvents Industry Alliance. My laboratory has published extensively on the biotransformation of TCE and was among the first to report formation of glutathione-*S*-conjugates from TCE. My area of expertise is biotransformation of xenobiotics, mechanisms of toxicity, and genotoxicity testing and I have published more then 180 manuscripts in these areas. Moreover, I am/was member of several advisory panels charged with health risk assessment of chemicals including the European Union Scientific advisory committee on Health and Environment (SCHER). As a member of this committee, I was the lead author of the review of the European Chemicals Bureau risks assessment report on TCE. I also have followed the many controversies in the risk assessment of TCE over the last 30 years.

General comments

The toxicity database on TCE is very large, with a number of controversial areas relevant to health risk assessment. EPA has generated a large document and attempted to comprehensively cover the available toxicology information on TCE and its metabolites. Most of the available studies are covered by the assessment. However, the document would have benefited from a detailed evaluation of the strengths and weaknesses of the individual studies and a selection of key studies based on a weight of evidence approach. In several places in the document, study results are just reiterated and some of the conclusions relevant for deriving RfDs and RfCs have apparently been taken from reviews. A detailed justification based on evaluation of the individual studies and a consideration of controversial data not supporting conclusions by EPA is often insufficiently developed. Identical criteria should be applied to the level of evidence required to support or discount a mode of action (MoA).

Specific comments:

1. Extent of glutathione *S*-conjugate formation from TCE

The document concludes that the extent of formation of *S*-(1,2-dichlorovinyl)glutathione (DCVG) from TCE in humans is much higher as compared to rodents. Since this conclusion has a major impact on the derivation of RfCs and RfDs for TCE, it should be well justified and based on consideration of all available data. Apparently, EPA supports

this conclusion with high blood concentrations of DCVG reported in humans after inhalation of TCE (Lash *et al.*, 1999b). This observation is in contrast to the very low concentrations of the isomers of N-acetyl-S-(1,2-dichlorovinly)-L-cysteine (N-acetyl-DCVC) in urine. The consideration of this dataset without the wealth of other information therefore suggests that which therefore can not be a quantitative biomarker of metabolic flux through the glutathione conjugation pathway (Lash *et al.*, 2000) and that most of the DCVG may undergo bioactivation by ß-lyase. However, a number of observations do not support this conclusion:

- In the human study with TCE inhalation, high concentrations of DCVG were indicated using a complex analytical procedure, often called the "Reed-Method" (Reed *et al.*, 1980). This method was developed to determine low concentrations of glutathione and glutathione disulfide and may be used to quantify DCVG formation in biological samples. The method involves reaction of the thiol with iodoacetamide and the amino group with chlorodinitrobenzene, followed by ion exchange chromatography and UV-detection of the dinitrophenyl chromophore. Due to the ion-exchange chromatography with a high salt concentration in the eluate, retention times shifts are common due to column deterioration (Lash *et al.*, 1999b). Since the method is not selective for DCVG and analysis of biological samples produces many peaks, retention time shifts may create problems to locate the DCVG peak.

A number of inconsistent datasets questions the reliability of the "Reed-method" to determine DCVG and DCVC:

- In a study assessing DCVG and DCVC formation in rodents after high oral doses of TCE, DCVG-concentrations reported in blood were high, but did not show dose or time-dependence (Lash *et al.*, 2006). In addition, the study reports high concentrations of DCVC excreted in urine. EPA calls the results of this study "aberrant", but apparently did not further assess reliability. Others have reported a very low rate of DCVC-formation in vivo (Dekant *et al.*, 1990; Kim *et al.*, 2009) and DCVC has not been reported as urinary metabolite of TCE using either mass spectrometry or HPLC which radiochemical detection after administration of ^{14}C-TCE (Dekant *et al.*, 1986a).
- The "Reed-method" has also been used to determine DCVG-formation from TCE in subcellular fractions from liver and kidney of rats, mice, and humans. Again, high rates of formation of DCVG were reported (table 1). In contrast, using ^{14}C-TCE and radioactivity detection, much lower reaction rates were observed in other studies (table 1). In addition, isolated glutathione *S*-transferases also have a very low capacity to metabolize TCE to DCVG (Hissink *et al.*, 2002) and the application of the "Reed-method" to study formation of *S*-(1,2,2-trichlorovinyl)glutathione (TCVG) from perchloroethylene in subcellular fractions also gave much higher rates of formation (Lash *et al.*, 1998) as compared to methods using ^{14}C-perchloroethylene and HPLC with radioactivity detection (Dekant *et al.*, 1987; Green *et al.*, 1990; Dekant *et al.*, 1998).

Therefore, DCVG concentrations determined by the "Reed-method" may be widely overestimated. The more reliable and consistent data support a very low extent of DCVG formation in rodents:

- Very low rates of formation of DCVG in rodents liver subcellular fractions are consistent with very low blood levels of DCVG in mice (Kim *et al.*, 2009) and a very low biliary elimination of DCVG in rats after oral administration of doses > 2 000 mg TCE/kg bw (Dekant *et al.*, 1990). In mice, DCVG concentrations were several 1,000-fold lower than those of the oxidative metabolite trichloroacetic acid (TCA) (Kim *et al.*, 2009). In rats, biliary elimination of DCVG within seven hours after oral administration was 2 microg and accounted for << 0.01 % of administered dose (Dekant *et al.*, 1990). Due to its

molecular weight (> 350 D) and the presence of effective transport systems for glutathione *S*-conjugates in the canalicular membrane, most of the DCVG formed in rat liver is expected to be excreted with bile. Therefore, the low concentrations of DCVG in blood of mice and the low recovery of DCVG in bile of rats after TCE-administration well support very low rates of DCVG formation.

- Even when considering the high rates of DCVG formation reported in subcellular fractions and the only 3-fold difference in reaction rates between mouse, rat and humans (table 1), it is difficult to explain why DCVG-blood levels in mice after a very high oral dose are orders of magnitude lower than those reported in humans after inhalation exposures giving a much lower internal TCE-dose.
- High blood concentrations of DCVG and a high flux through ß-lyase bioactivation are not consistent with the human toxicity data on TCE. Despite high occupational exposures to TCE between the 1950s and 1970s (occupational exposure limits for TCE were 200 ppm in Germany and were often exceeded for prolonged times), overt nephrotoxicity was rarely observed even after many years of exposures (MAK, 1996). Using the blood concentrations reported and extrapolating to a daily exposure to 200 ppm TCE for 8 h, daily doses of DCVC of app. 5-7 mg/kg bw should have been received by workers. A significant flux through ß-lyase bioactivation should have resulted in renal effects considering the alleged potency of DCVG.
- Kinetic studies on acetylation, and ß-lyase-mediated metabolism of DCVC support a low flux through ß-lyase activation since the relative flux through the *N*-acetylation pathway (detoxication) is one to two orders of magnitude higher then through ß-lyase activation (Green *et al.*, 1997a). In addition, a low flux through ß-lyase is indicated by the recovery of most of a low intravenous dose of DCVC isomers in urine as mercapturic acids in rats (Birner *et al.*, 1997), the weak nephrotoxicity of DCVC (Green *et al.*, 1997a) and observations with perchloroethene, which is also metabolized by glutathione *S*-conjugate formation and ß-lyase. The perchloroethylene (PERC) metabolite *S*-(1,2,2-trichlorovinyl)-L-cysteine is cleaved by ß-lyase to dichloroacetic acid (DCA) which, when formed in the kidney, is excreted with urine. While DCA is a metabolite of PERC in rats, this compound is not excreted as PERC metabolite in humans (Völkel *et al.*, 1998). In addition, dichloroacetylated proteins were detected both in rat kidney proteins and rat blood proteins after PERC inhalation. Such protein modifications were not detected in blood proteins from humans after identical exposures (Pähler *et al.*, 1999). These observations indicate that flux through ß-lyase in humans is even lower as compared to rodents.
- Chloracetic acid is formed by ß-lyase from DCVC (Dekant *et al.*, 1988). In rodents, chloroacetic acid and its metabolites (Green and Hathway, 1975; Green and Hathway, 1977) are not significant metabolites of TCE (> 0.1 % of radioactivity in urine) (Dekant *et al.*, 1984; Dekant *et al.*, 1986a). If the ß-lyase pathway is more relevant, such metabolites should be present in urine in higher concentrations. Other metabolites indicative of alternative processing of DCVC have also not been detected in humans (Bloemen *et al.*, 2001).

In summary, the assumption of a major flux through glutathione S-conjugate formation in TCE metabolism both in humans and in rodents is not well supported.

Table 1: Reported rates of formation of DCVC from Trichloroethene (TCE) in rat, mouse and human subcellular fractions. The concentration of TCE in the incubation is based on the amount added.

Tissue	Species	TCE Conc (mM)	Rate of DCVC formation (pmol/minxmg)	Analytical method to determine DCVG	Reference
Liver cytosol	Rat	1.4 (14C)	0.54 (non-enzymatic reaction rates substracted)	HPLC with radiochemical detection, peak identity confirmed by LC/MS	(Green *et al.*, 1997b)
	Mouse	1.9 (14C)	0.35		
	Human	1.9 – 2.5 (14C)	0.012 – 0.055		
Liver microsomes	Rat	1.4 (14C)	Not different from non-enzymatic reaction		
	Mouse	1.9 (14C)	n.d.		
	Human	1.9 – 2.5 (14C)	n.d.		
Kidney cytosol	Rat	1.4 (14C)	Not different from non-enzymatic reaction		
	Mouse	n.d.			
	Human	n.d.			
Kidney microsomes	Rat	1.4 (14C)	Not different from non-enzymatic reaction		
	Mouse	n.d.			
	Human	n.d.			
Liver cytosol	Rat	4 (14 C)	< 2	HPLC with radioactivity detection, peak identity confirmed by GC/MS after hydrolysis	(Dekant *et al.*, 1990)
Liver microsomes	Rat	4 (14C)	2		
Liver cytosol	Rat	2	121 (males) 81 (females)	Derivatisation with DNCB and ion exchange HPLC	(Lash *et al.*, 1999a)
	Mouse	2	408 (males) 361 (females)		
	Human	1	1 700 – 4 180		
Liver microsomes	Rat	2	171 (males) 120 (females)		
	Mouse	2	666 (males) 426 (females)		
	Human	1	495 – 3 245		
Kidney cytosol	Rat	2	7.5 (males) 5.3 (females)		
	Mouse	2	93 (males) 61 (females)		
	Human	na	810 (vmax)		
Kidney microsomes	Rat	2	Nd (males) 1.0 (females)		
	Mouse	2	91 (males) 278 (females)		
	Human	na	6 290 (vmax)		

2. The role of glutathione S-conjugates in nephrotoxicity and renal tumor formation by TCE

Since *S*-conjugates of TCE are nephrotoxic in rodents and genotoxic in vitro, it is appealing to conclude that *S*-conjugate formation is involved in nephrotoxicity of TCE and that the MoA for kidney tumor formation is genotoxicity. However, a number of contradictory findings are not adequately considered in the IRIS-document.

- Formation rates for DCVC in subcellular fractions from mice and rats are similar (or even higher in mice) suggesting similar doses of DCVC to the kidney in both species (Green *et al.*, 1997a; Kim *et al.*, 2009). Moreover, activation of TCE by the ß-lyase pathway is higher in mice (Eyre *et al.*, 1995), DCVC is more nephrotoxic in mice, and causes higher rates of cell replication and covalent binding in mice as compared to rats (Eyre *et al.*, 1995; Green *et al.*, 1997a). Yet, mice are not sensitive to TCE induced renal tumor formation.
- Based on the nephrotoxicity of DCVC and the low rates of formation of DCVC both in rats and mice in vivo, it is questionable if the very low concentrations of DCVG formed in rodents can explain nephrotoxicity and tumor formation. Extrapolating the DCVG blood concentrations observed after single doses to the doses applied in the carcinogenicity studies, daily DCVC-doses in the two year studies were less than 0.03 mg/kg bw. This is orders of magnitude below the doses of DCVC required to induce nephrotoxicity (Terracini and Parker, 1965) and questions an involvement of this pathway in nephrotoxicity.
- EPA concludes that trichloroethanol and formic acid formation may not be involved in the toxicity of TCE to the kidney due to differences in pathology observed between TCE and trichloroethanol treated rats. In my opinion, such comparisons are difficult since differences in the kinetic profiles of a compound formed as a metabolite or administered per se are likely major confounders.
- EPA states that data on VHL gene mutations support a mutagenic MoA in TCE-induced kidney tumors. This is based on studies (Bruning *et al.*, 1997; Brauch *et al.*, 2004) reporting VHL mutations in renal tumors of TCE-exposed individuals. It is concluded that comparison of TCE-exposed and non-exposed patients (Brauch *et al.*, 2004) revealed clear differences with respect to (1) frequency of somatic VHL mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. As discussed in Brauch et al. (2004), the mutation frequency in the non-exposed patients (10%) was considerably lower than that commonly observed in sporadic renal tumors, e.g. 82.4% (Nickerson *et al.*, 2008) or 71% in (Banks *et al.*, 2006), and technical problems using archived tissue samples may be the cause. Given that exon 3, which harbors the multiple mutations seen in TCE exposed patients, did not amplify in most of the controls, there is limited evidence for a difference in the incidence of multiple mutations and frequency of somatic VHL mutations, although the C454T transition appears to be characteristic of tumors in TCE exposed patients. However, the presence of mutations in human tumors does not lead to the conclusion that VHL mutations occur early during carcinogenesis and hence are no evidence for direct genotoxicity of TCE. In contrast, experimental data in rats show that neither TCE nor its active metabolite DCVC induce VHL mutations (Mally *et al.*, 2006), suggesting that VHL mutations in humans may be acquired at later stages of tumor development. While the document argues that the VHL gene may not be a target gene in rodent models of renal carcinogenesis, only few studies have looked at VHL in rats and there is no support for the hypothesis that the role of VHL is different in rats and humans.

- The Eker rat may be an useful rodent model for renal cell carcinoma (RCC), but the molecular basis for chemically induced tumor formation in rats and RCC in humans may be widely different from spontaneous tumor formation in this rat strain, as high-grade RCCs can develop in the absence of mutations in the Tsc2 gene in rats (Toyokuni *et al.*, 1998). Development of high-grade renal cell carcinomas in rats independently of somatic mutations in the Tsc2 and VHL tumor suppressor genes (Toyokuni *et al.*, 1998) demonstrates that mutational inactivation of TSC2 or VHL is not a prerequisite for renal carcinogenesis. The similar pathway activation in Eker rat RCC as that seen in humans with VHL mutations reported (Liu *et al.*, 2003) involves deregulation of HIFalpha and VEGF expression which frequently occur in various cancers and provide little evidence to suggest that Tsc-2 inactivation in rats is "analogous" to inactivation of VHL in human RCC.
- Epidemiological data may support an association between specific VHL mutations and TCE exposure, this does not indicate an early event in RCC and – in the absence of experimental support - should not be taken as support for a mutational MoA.
- EPA uses a micronucleus/comet assays data in rat kidney after TCE-administration as support for a genotoxic MoA. However, the positive micronucleus (Robbiano *et al.*, 2004) assay applied a very high dose and used an inappropriate route of administration (ip injection of ½ of the LD_{50}). Due to the high dose applied and the route of administration, the results may be confounded by inflammatory responses and should not be used for conclusions. A comet assay in the kidney using repeated inhalation exposures to TCE was negative (Clay, 2008). The decision to not use this study in the assessment is insufficiently justified. The inhalation study used a higher number of animals (5/group) as compared to the ip study, which states n > 3 with an apparent maximum of 5. The comet assay also shows that administered DCVC is only weakly active in the kidney.
- EPA argues that there is no link between nephrotoxicity and renal tumor formation. However, there are a number of compounds causing renal tumors in rats without being genotoxic. For example, cytotoxicity and regenerative cell proliferation (Swenberg and Lehman-McKeeman, 1999) is accepted as MoA for α_{2u}-globulin binding agents (TCE does not bind to α_{2u}-globulin, but may also causes tumors through nephroxicity).

3. Mode of action for liver carcinogenesis

- EPA spends considerable effort to correlate liver tumor induction by TCE in mice with liver tumor induction observed after administration of the TCE metabolites TCA and DCA. Again, such comparisons are inherently complex. Both DCA and TCA were administered with drinking water and TCE studies applied gavage in oil. The different administration regimens will result in different time courses of the administered compounds or metabolites in blood and dose-dependent bioavailability may further complicate the interpretation.
- It is highly questionable that DCA is involved in liver tumor induction by TCE since it is only formed in very low concentrations from TCE in rodents (Dekant *et al.*, 1986a; Kim *et al.*, 2009). In mice, DCA is formed in concentrations several orders of magnitude below those of TCA. Thus, DCA would be required to be a highly potent liver carcinogen, which it is not. Therefore, the potency data on DCA do not suggest that the high liver tumor incidence induced by TCE in mice is related to DCA formation. In

addition, DCA is not a human urinary metabolite of TCE (Bernauer *et al.*, 1996; Bloemen *et al.*, 2001).

- With TCA, EPA derives a dose-dependence from tumor incidence data in drinking water studies. Apparently, EPA assumes a dose-independent high bioavailability of TCA. However, the oral bioavailability of TCA from drinking water is limited, concentration-dependent and significantly reduced at higher concentrations of TCA (Larson and Bull, 1992; Templin *et al.*, 1993; Sweeney *et al.*, 2009). The incidence data therefore need to be corrected to account for the limited bioavailability of TCA at higher concentrations in drinking water.
- The mostly negative data in mutagenicity testing with TCE using liver specific activation and negative in vivo gentoxicity data including a very low DNA-binding in liver of mice (Bergman, 1983; Kautiainen *et al.*, 1997) also do not support a mutagenic MoA for liver tumors. Due to intensive metabolism by oxidation and reduction, chloral hydrate concentrations in the liver are low, chloral hydrate is a very weak mutagen. Therefore, chloral hydrate mutagenicity cannot adequately explain the formation of liver tumors by TCE in mice.
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4. Mode of action for lung tumorigenesis.

EPA considers the lung tumors induced by TCE in specific strains of mice as relevant to humans and implies a genotoxic mode-of action. EPA tries to devaluate the hypothesis that chloral may reach high concentrations in mouse lung cells. However, the arguments by EPA are not convincing.

Rat and guinea pig data should not be used to conclude on biotransformation in mouse lung.

- A delivery of TCE from the systemic circulation in mice also causes lung toxicity due to the high metabolic capacity in the target cell. If TCE-metabolites formed in the liver are transported to the lung to cause toxicity there, the species-specificity is difficult to explain since the same metabolites are also present in rats, which do not show lung toxicity.
- A high rate of chloral formation from TCE and limited capacity for further metabolism of chloral (low capacity for reduction of chloral hydrate to trichloroethanol, low capacity for conjugation of trichloroethanol) will result in much higher steady state levels of chloral hydrate in mouse lung Clara cells as compared to rat or human lung (Odum *et al.*, 1992; Green *et al.*, 1997b). The high steady state levels may result in cytotoxicity.
- Cells damaged by the high chloral concentrations formed by TCE-metabolism initiate regeneration and replication to repair and replace the damaged Clara cells (Villaschi *et al.*, 1991) and repeated cycles of damage and regeneration may finally result in lung tumor formation.

Support for a cytotoxic MoA regarding the mouse lung tumors induced by TCE can also be derived from observations with other chemicals. The consequences of Clara cell specific cytotoxicity for tumor induction has been assessed with a number of other chemicals and the very high capacity of the mouse lung Clara cell for biotransformation is also the basis for the mouse-specific lung toxicity. The assessment therefore should integrate this information.

- Styrene, naphthalene, and coumarin induce lung tumors in mice and chronic damage of Clara cells including hyperplasia, often with a time- and dose-related increase in

bronchiolar hyperplasia in terminal bronchioles. As with TCE, lung lesions are induced by short term administration, recess after repeated exposures and reappear after continuing exposures. None of these chemical induced lung tumors or histopathologic changes in rat lung (Cruzan *et al.*, 1998; Cruzan *et al.*, 2001).

- Major species differences in lung tumor induction and lung anatomy are one likely basis for the selective tumorigenicity of these chemicals in mice. Lung tumors occur spontaneously in several mouse strains and the incidences of benign lung tumors in control mice are often very high. In general, murine lung tumors are mostly adenomas originating from bronchiolar Clara cells. The adenomas may progress to adenocarcinomas. (Witschi, 1991).
- *Clara cells are the major site of xenobiotic metabolism in the mouse lung* (Chichester *et al.*, 1991; Buckpitt *et al.*, 1995). In addition to marked species differences in metabolic capacity of Clara cells in different species, species differences in Clara cell abundance and function may contribute to selective pulmonary toxicity in mice. Clara cell number is significantly higher within the terminal bronchioles of mice relative to rats and humans (Plopper *et al.*, 1980; Lumsden *et al.*, 1984). Clara cells represent approximately 5 % of all cell types and are distributed throughout the airways in mice. In humans, only very few Clara cells are present and are localized in specific regions. Moreover, Clara cells differ morphologically among species, with human cells containing little smooth endoplasmic reticulum.
- TCE and the other chemicals inducing selective lung damage and lung tumors in mice require biotransformation by pulmonary CYP2F and CYP2E1 (Green *et al.*, 1997b; Shultz *et al.*, 1999; Shultz *et al.*, 2001; Born *et al.*, 2002; West *et al.*, 2002; Forkert *et al.*, 2005).
- In mice, both CYP2E1 and CYP2F1 are preferentially localized in Clara cells (Forkert *et al.*, 1989; Buckpitt *et al.*, 1995; Forkert, 1995; Shultz *et al.*, 2001). In rat lung, the *expression of CYP2F4, an orthologe of mouse CYP2F2* (Baldwin *et al.*, 2004) is app. 30-fold lower consistent with a much lower turnover of CYP2F substrates in rat. Evidence for the presence of the the human orthologe CYP2F1 in human lung is lacking. In rhesus monkeys, CYP2F1 was not detected in the respiratory tract except in the nasal epithelium (Ding and Kaminsky, 2003; Baldwin *et al.*, 2004). CYP2E1 catalytic activity is present in human lung with an activity app. 100fold lower then in human liver (Bernauer *et al.*, 2006). In summary, the available information on the presence and catalytic activities of CYP2E1 and CYP2F enzymes in the lung of different species suggest a much higher activity of these enzymes in the mouse, the species susceptible to the pneumotoxicity.
- Studies directly quantifying relevant metabolite formation from the different pneumotoxic compounds and mice consistently have a much higher capacity for oxidation as compared to rats and humans. The available data on the mode-of-action for induction of lung tumors share many common features with regard to the induction of Clara cell lesions in the mouse and a number of observations support a non-genotoxic mode-of-action: Glutathione depletion is a major determinant of the toxic responses in the mouse Clara toxicity (West *et al.*, 2000a; West *et al.*, 2000b; Plopper *et al.*, 2001; Phimister *et al.*, 2004; Turner *et al.*, 2005). Glutathione-depletion induced cell death induced by mouse specific Clara cell toxicants initiates extensive cell replication and subsequent hyperplasia which are considered important steps in the multi-step progression to tumor development (Gadberry *et al.*, 1996; Green *et al.*, 1997b; Green *et al.*, 2001).
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Additional comments

Page 2-22: Line 36, the exposures in the cardboard workers in Germany likely were much higher, with peaks well above 1,000 ppm and prolonged exposures above the former occupational standard (> 200 ppm TWA).

Page 3-6: The major toxicity of TCE after acute high dose exposure is narcosis. Both kidney and liver damage are not often observed (MAK, 1996).

Page 3-13: Table 3-6, if the data in the table are not considered reliable why are they presented?

Page 3-15: Line 27, TCA reversibly binds to proteins and the reversible protein binding is much more relevant for toxicokinetics of TCE as compared to covalent binding. It should also be noted that the ^{14}C-TCE used in many of the early studies contained a number of reactive impurities.

Page 3-23: Regarding saturation of TCE metabolism in humans, none of the human studies used dose-ranges where saturation of metabolism was seen in rats. Therefore, this conclusion should be removed.

Page 3-24: Lines 9 to 14, the text is not logical. TCE oxide may rearrange to dichloroacetyl chloride and the TCE P450 intermediate may rearrange to give chloral (Miller and Guengerich, 1982; Liebler and Guengerich, 1983; Cai and Guengerich, 2001).

Page 3-25: Lines 20 to 23, TCE oxide does not rearrange to chloral. Therefore, the text is confusing.

Page 3-27, Lines 19 to 25, chloral hydrate as been identified as a circulating TCE metabolite and is also formed as the major product in the microsomal oxidation of TCE (Byington and Leibman, 1965; Cole *et al.*, 1975).

Page 3-35: Metabolite recovery data in male and female human beings are available. In addition, metabolite excretion in humans and rats exposed under identical conditions are available (Bernauer *et al.*, 1996).

Page 3-44: Table 3-23 should include additional data on GSH-conjugation of TCE (Dekant *et al.*, 1990; Green *et al.*, 1997a).

Page 3-46: Information on ß-lyase catalyzed metabolism of DCVC is available (Green *et al.*, 1997a).

Page 3-47: DCVC-sulfoxide, it should be mentioned that sulfoxides and down-stream metabolites have never been directly identified in rodents.

Page 4-34: Line 1, conclusion on bacterial mutagenicity. A more detailed weight-of-evidence evaluation of the contradictory database is needed here.

Table 4-18: Robbiano study, the study did not apply DCVG or DCVC and thus should not be included in the table.

Page 4-83: Line 28, DCVC is a "direct-acting" mutagen since bacteria express ß-lyase (Dekant *et al.*, 1986b). Thus, this is a difference when compared to *S*-(2-chlorethyl)-L-cysteine, which does not require enzymatic transformation.

Page4-443: Lines 6 -7, the reactivity of chloral hydrate and chloroacetaldehyde are highly different and should not be compared. Chloroacetaldehyde is highly reactive with DNA-constituents (Green and Hathway, 1978), whereas chloral hydrate has not.

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APPENDIX 5

Peer Reviewer Comments on Draft TCE Work Plan Assessment[1]

It is clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan assessment that EPA carried out for TCE. There can be no doubt that this is the proper characterization of the June 2014 assessment. The Chairperson of EPA's peer review panel wrote:

> "The draft document fails to articulate satisfactorily that the analysis described within should be characterized as a screening level assessment. . . . I believe that the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . .
>
> "After listening carefully to the comments and contributions from the other members of the Panel, I have concluded that there would little benefit in revising this draft screening assessment. Rather, I would suggest that the effort be put into a higher tier, more refined assessment which would include empirical data gathered during the course of real-world uses, e.g., as OPP regularly asks be done for occupational exposures and sometimes for residential exposures, consumer use survey data, evaluation of exposure using additional modeling tools and a revisiting and reanalysis of the choices of toxicity and epidemiologic studies used to describe the health benchmark at the MEC99 level and the rationale for selecting the singular MOE of 30 to apply to the selected studies, each of which have varying degrees of credibility. This current draft screening level assessment could then be attached as an appendix to the new second-generation assessment, and described, in summary form, in the early chapter(s) of the new assessment. I would have saved the resources expended for the current external peer review and spent them on the next-generation assessment."
>
> She further stated:
>
> "By selecting the HEC99 and very conservative assumptions about exposure, one ends up with a very conservative (that is, health-protective) risk assessment, which assures only the certainty that the potential risk has not been under-estimated. It does little to resolve the uncertainty of the true estimate of risk."

The Chairperson's main point was that the information (*i.e.*, the screening level assessment) is not consistent with any intended use to support regulation. Her advice was that there would be little benefit in even revising the assessment, given its inadequacy for regulatory use. Taken together, these comments by the Chairperson of EPA's peer review panel establish quite clearly that the TCE risk evaluation does not meet the requirements of new TSCA § 26(h).

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[1] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

One of the peer review panelists, Calvin Willhite, raised serious concerns over the derivation of the non-cancer dose-response:

> "The non-cancer hazard index not only leads to calculation of the lowest equivalent 'safe' concentration of TCE in residential air, but those values are either less than or consistent with background TCE concentrations in United States urban or residential indoor air. As such, any domestic use of TCE in any amount for any use whatsoever will exceed the US EPA's published residential indoor air TCE level (0.21 μg/m3). As written, the previously published and current US EPA reports lead to the conclusion that current ambient TCE levels are associated with increased risk for human cardiovascular malformations - yet there are no suggestions from studies of occupational TCE exposures at concentrations 1-2 magnitude of orders greater than ambient pose excess non-cancer health risks to those workers."

With regard to uncertainty, weight of scientific evidence, quality and reproducibility, and other criteria identified in § 26(h), Dr. Willhite stated:

> "Question 5-4. Please comment on whether the document has adequately described the uncertainties and data limitations. Please comment on whether this information is presented in a transparent manner.
>
> "The general comments concerning the OPPT and IRIS conclusions on risk for cardiovascular malformations above illustrate the poor weight of evidence assessment carried out in this regard for TCE. The uncertainty attendant to the IRIS hazard identification for cardiovascular terata is so great that it leads to the present OPPT conclusion that all TCE exposures (including background concentrations in US urban ambient and indoor residential air) present increased risk for congenital malformation of the heart and great vessels.
>
> "It is not clear why OPPT relied on the results of the Johnson et al. (2003) study to the exclusion of all other inhalation and oral developmental toxicity studies in rodents and rabbits. If in fact the OPPT is reliant upon only the inhalation data, why is it the Carney et al. (2001), the Schwetz et al. (1975), the Hardin et al. (1981), the Beliles et al. (1980) or the Dorfmueller et al. (1979) study was not used? Why is there no discussion of all of the available developmental toxicity inhalation bioassays in the present analysis?
>
> "Summary
>
> "As submitted, the exposure parameters appear arbitrary (e.g., 0.5 and 1 hr/day) and may have been selected for sake of convenience. The data upon which conclusions put forward by OPPT on risk for developmental toxicity associated with arts and crafts use of TCE are not reliable. Nearly all developmental toxicity studies with TCE in rodents find no sign of teratogenicity (e.g., Beliles et al., 1980) or find only slight developmental delay (Dormueller et al., 1979). Chiu et al. (2013) cite the NRC (2006) report as verification of their risk assessment for TCE developmental toxicity, but actually the NRC (2006) concluded:

> 'Additional studies evaluating the lowest-observed-adverse- effect-level and mode of action for TCE-induced developmental effects are needed to determine the most appropriate species for human modeling.'

"In its present assessment, the OPPT ignored the serious deficiencies already identified in conduct of the Johnson et al. (2003) rat drinking water study upon which the BMD01 was based (Kimmel et al., 2009; Watson et al., 2006) [Attachments 1 and 2]. In their weight-of-evidence assessment, Watson et al. (2006) concluded:

> '...application of Hill's causality guidelines to the collective body of data revealed no indication of a causal link between gestational TCE exposure at environmentally relevant concentrations and congenital heart defects.'

"Those conclusions were consistent with Hardin et al. (2005). Perhaps most disturbing of all in US EPA's reliance upon Johnson et al. (2003) as the key study (which for the basis for their lowest non-cancer TCE hazard index and margin of exposure) is the observation by Hardin and associates (2004):

'Conventional developmental and reproductive toxicology assays in mice, rats and rabbits consistently fail to find adverse effects of TCE on fertility or embryonic development aside from embryo- or fetotoxicity associated with maternal toxicity. Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a 'specific' cardiac teratogen.'

"One of the fundamental tenants in science is the reliability and reproducibility of results of scientific investigations. In this regard, one of the most damning of the TCE developmental toxicity studies in rats is that by Fisher et al. (2005) who stated:

'The objective of this study was to orally treat pregnant CDR(CD) Sprague-Dawley rats with large bolus doses of either TCE (500 mg/kg), TCA (300 mg/kg) or DCA (300 mg/kg) once per day on days 6 through 15 of gestation to determine the effectiveness of these materials to induce cardiac defects in the fetus. All-trans-retinoic acid (RA) dissolved in soybean oil was used as a positive control.

"The heart malformation incidence for fetuses in the TCE-, TCA- and DCA-treated dams did not differ from control values on a per fetus or per litter basis. The RA treatment group was significantly higher with 33% of the fetuses displaying heart defects.'

"Unfortunately, Johnson et al. (2005) failed to report the source or age of their animals, their husbandry or provide comprehensive historical control data for spontaneous cardiovascular malformations in their colony. The Johnson study with 55 control litters compared to 4 affected litters of 9 treated was apparently conducted over a prolonged period of time (perhaps years); it is possible this was due to the time required to dissect and inspect fresh rodent fetuses by a small

academic research group. However, rodent background rates for malformations, anomalies and variants show temporal fluctuations (WHO, 1984) and it is not clear whether the changes reported by Johnson et al. (2005) were due to those fluctuations or to other factors. Surveys of spontaneous rates of terata in rats and other laboratory animals are common particularly in pharmaceutical and contract laboratory safety assessment (e.g., Fritz et al., 1978; Grauwiler, 1969; Palmer, 1972; Perraud, 1976). The World Health Organization (1984) advised:

> 'Control values should be collected and permanently recorded. They provide qualitative assurance of the nature of spontaneous malformations that occur in control populations. Such records also monitor the ability of the investigator to detect various subtle structural changes that occur in a variety of organ systems.'

"Rates of spontaneous congenital defects in rodents can vary with temperature and housing conditions. For example, depending on the laboratory levocardia and cardiac hypertrophy occur in rats at background rates between 0.8-1.25% (Perraud, 1976). Laboratory conditions can also influence study outcome; for instance, maternal hyperthermia (as a result of ambient elevated temperature or infection) can induce congenital defects (including cardiovascular malformations) in rodents and it acts synergistically with other agents (Aoyama et al., 2002; Edwards, 1986; Zinskin and Morrissey, 2011). Thus while the anatomical observations made by Johnson et al. (2003) may be accurate, in the absence of data on maternal well-being (including body weight gain), study details (including investigator blind evaluations), laboratory conditions, positive controls and historical rates of cardiac terata in the colony it is not possible to discern the reason(s) for the unconventional protocol, the odd dose-response and marked differences between the Johnson et al. (2003) results and those of other groups.

"As noted by previous investigators, the rat fetus is "clearly at risk both to parent TCE and its TCA metabolite" given sufficiently high prenatal TCE exposures that can induce neurobehavioral deficits (Fisher et al., 1999; Taylor et al., 1985), but to focus on cardiac terata limited to studies in one laboratory that have not been reproduced in other (higher dose) studies and apply the BMD01 with additional default toxicodynamic uncertainty factors appears misleading."

Finally, Michael Jayjock, another peer review panelist, concluded: "Clearly, more work is needed on both the exposure and hazard side of this evaluation to tighten up the exposure assessment and to provide further justification or explanation of the exceedingly low HEC99 values used in the MOE analysis."

As discussed above, other panelists raised serious concerns going to the heart of the "best available science" criteria in TSCA § 26(h). Peer review and public comments identified numerous scientific deficiencies with the draft TCE assessment, including the inappropriate use of default assumptions; ignoring contrary evidence that affects the weight of the scientific evidence; reliance on inapposite exposure data; conclusions inconsistent with the evidence cited;

and, most importantly, reliance on a study that is not reproducible. Equally important deficiencies in both the hazard and exposure assessments were noted.

EPA completely disregarded the peer reviewers' advice and issued the final Work Plan assessment in June 2014 without making any substantial change to the draft. Under TSCA § 26(h), however, EPA must make its science-based decisions "in a manner consistent with the best available science" and "based on the weight of the scientific evidence." In addition, EPA can no longer afford to ignore the conclusions of the peer review it initiated, as it must consider "the extent of independent verification or peer review of the information."

APPENDIX 1 (CORRECTED)

HSIA halogenated solvents industry alliance, inc.

MEMORANDUM

To: Docket EPA-HQ-OPPT-2016-0163

From: Faye Graul
Executive Director

Date: March 16, 2017

Subj: Trichloroethylene Drinking Water Study

Attached please find a copy of Protocol Number WIL-459501 titled An Oral (Drinking Water) Study of the Effects of Trichloroethylene (TCE) on Fetal Heart Development in Sprague Dawley Rats. The Protocol was signed on October 6, 2016 and the in-life portion of the study was conducted during October and November, 2016. Unfortunately, the concentrations of TCE measured in the drinking water solutions were found to be below the acceptable target range of 100% ± 10%, invalidating the study. The laboratory is conducting additional studies to identify the source of the deviations and the study will be rerun as soon as the dosing methodological issues are resolved and scheduling permits.

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WIL RESEARCH

5 August 2016
Proposal: 15.04279

Proposal for Halogenated Solvents Industry Alliance

Proposal provided by:
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WIL RESEARCH

Proposal Summary

Study	Base Study Fee	Optional			Total Study Fee	
		AC	BioAC	TK Report		Authorized
A (5-Group) Prenatal Developmental Toxicity Study of TCE Administered by Drinking Water in Sprague Dawley Rats	$168,000	$12,400	$15,730	$4,100	$200,230	☒
Analytical Validation, Homogeneity, and Stability Study of the Analyte in Aqueous Formulations	$24,160	-	-	-	$24,160	☒
Development and Testing of an LC-MS/MS Method for the Quantification of Test Article (TCE) and a Major Metabolite (TCA) in Rat Plasma	$11,050	-	-	-	$11,050	☒
Validation of an LC-MS/MS Method for the Quantification of Test Article in Rat Plasma	$28,750	-	-	-	$28,750	☒

***Fee and Payment Schedules are subject to credit approval.**

Authorization Statement

Halogenated Solvents Industry Alliance ("Sponsor") hereby awards the above described proposal (the "Proposal") to WIL Research Laboratories, LLC ("WIL") (each a "Party"), and requests WIL to proceed with the necessary activities to initiate these Services, including but not limited to, Protocol development, Study room reservation, and definitive scheduling of Services.

This Proposal, the performance of Services, and each Party's obligations herein are governed by and subject to the WIL Research Laboratories LLC General Terms and Conditions attached hereto (the "General Terms and Conditions"). The General Terms and Conditions are hereby incorporated by reference to this Proposal in their entirety. By executing below, Sponsor acknowledges and represents, and the undersigned person executing this Proposal on behalf of Sponsor certifies, that such person has read and Sponsor agrees to the provisions set forth in the General Terms and Conditions.

This Proposal (including the relevant Protocol), together with the General Terms and Conditions and the Confidentiality Agreement between the Parties dated [08/08/16], constitutes the entire agreement (the "Agreement") between the Parties with respect to the subject matter contained herein. There are no oral or written promises, terms, conditions, or obligations other than those contained in this Agreement. This Agreement supersedes all prior negotiations, representations or other agreements, either written or oral, between the Parties on the subject matter related herein. No modification or waiver of the provisions of this Proposal, the General Terms and Conditions or the Confidentiality Agreement shall be valid or binding on either Party unless agreed to in writing by each Party.

In the event the terms of this Proposal or any other agreement between the parties hereto contradict any provision of the General Terms and Conditions, the General Terms and Conditions shall control unless expressly agreed to in writing by each Party herein.

Any notices given hereunder shall be sent by fax or email, with a confirmation copy sent via overnight courier to the following addresses (or such other address as a party may designate as a notice address in a written notice to the other party) and shall be deemed received when delivered (or if received on a weekend or holiday, on the next business day thereafter) as follows:

WIL RESEARCH

If to Sponsor: Name: John Bell
Title: Director, Scientific Programs
Company: Halogenated Solvents Industry Alliance, Inc.
Address: 3033 Wilson Boulevard
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By executing this document Sponsor understands, acknowledges and agrees to the financial responsibility for all costs and expenses in accordance with this Proposal including those incurred by WIL in preparation of the Study. Any modification that requires an increase in cost subsequent from the effective date of this Proposal will be adjusted through a Study Modification.

Signature of Authorized Sponsor Representative

August 8, 2016
Date

Name: John Bell, Ph.D., DABT

Title: Director, Scientific Programs

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Arlington, VA 22201

Email Address (invoices will only be sent as a PDF to this email address):

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A (5-Group) Prenatal Developmental Toxicity Study of TCE Administered by Drinking Water in Sprague Dawley Rats
Compliance: GLP, OECD
Guidelines: Modified OECD 414

Group	Toxicology Animals (145)	
	Toxicology Females (150)	Maternal TK (20)
1	25	4
2	25	4
3	25	4
4	25	4
5	25	4
6	25	-

Objective: To detect potential adverse effects on the pregnant female and on the development of the embryo and fetus consequent to exposure of the female starting the day after mating (Gestation Day 1) through implantation and gestation until one day prior to expected parturition.

Animals[2]: Female Sprague Dawley Rats Crl:CD(SD)
170 animals on study, 212 animals ordered

Untreated sexually mature males of the same strain and source will be used to induce pregnancies.

Groups: 1 control group, 4 test article-treated groups and 1 positive control group.

Dose Levels[3]: Highest dose will be 1100 ppm in drinking water based on a previous study conducted by Johnson et al.

Test Substance Preparation: Prepared at a frequency consistent with established stability.

Sampling of Formulations: From the first and last preparations. Samples analyzed at WIL Research (optional).

Test Substance Administration: Via drinking water (glass water bottles) from gestation day 1 until the day of scheduled necropsy at the end of gestation, inclusively. Day evidence of mating is confirmed is gestation day 0.

Group 6 (positive control group) dosed via oral gavage from Gestation Day 6-15, inclusively.

Viability Observations: Twice daily observations for moribundity and mortality.

Clinical Observations: Once daily.

Body Weights: Toxicology Animals: Gestation days 0-20 (daily).

Toxicokinetic Animals: Gestation days 0-20 (daily).

Food Consumption: Toxicology Animals: Gestation days 0-20 (daily).

Toxicokinetic Animals: Not recorded.

Toxicokinetics: Maternal TK Phase – Blood samples collected from each dam on GD 8, GD 16 and again at the end of the administration period (GD 20) from 4 maternal toxicokinetic animals/group/time point at a single time point (60 maternal samples). Samples can be analyzed at WIL Research (optional).

Fetal TK – Immediately following the final maternal tk blood collection on GD 20, each dam will be euthanized and fetal blood will be collected from the umbilical vessel of each fetus and pooled by litter (20 pooled fetal samples). Samples can be analyzed at WIL Research (optional).

Scheduled Laparohysterectomy: Toxicokinetic Animals: Gestation day 20; Determination of pregnancy status only and fetal blood collected as required.

Toxicology Animals: Gestation day 20; Examination of uterine contents: determination of pregnancy status, gravid uterine weights, gross evaluation

	of placenta and count of corpora lutea, implantation sites, early and late resorptions and viable and nonviable fetuses. Fetal Observations: External and fresh visceral examinations of all viable fetuses for developmental variations and malformations, sex ratios and body weights. The carcass of each fetus will be preserved and retained for possible future skeletal evaluation.
Quality Assurance:	The study will be conducted in compliance with Good Laboratory Practice (GLP) standards and will be monitored by the Quality Assurance Unit.
Reports:	Audited Draft Report and Final Report.
Archiving:	For a period of six months after study completion.

5-Group Base Study Fee (Full Fetal Visceral Evaluations) [1] $168,000

Optional Support Fees:

Analytical Chemistry (AC): [2] $3,400/set

Concentration determination (1st preparation with concurrent homogeneity):	$3,400
Resuspension homogeneity (1 interval):	$3,400
Concentration determination (last preparation):	$3,400
Sample analysis report:	$2,200
Total Study-specific AC:	$12,400

Bioanalytical Chemistry (BioAC): [4]

Sample analysis - 80 samples @ $85/sample (minimum batch 100 samples):	$8,500
Dilution repeats - 30 samples @ $85/sample[5] (estimated 10% of samples; minimum batch 30 samples):	$2,550
Incurred sample reanalysis - 8 samples @ $85/sample:	$680
Report Fee[6]:	$4,000
Total Study-specific BioAC:	$15,730

Toxicokinetic Report:

Preparation of a toxicokinetic report from the maternal and fetal exposure data for a single analyte and single dose route. Preliminary toxicokinetic results will be available upon request and will typically be provided within two business days of availability of bioanalytical data.

TK Report: $4,100

1. Final price depends on the technical details in the final protocol and will be set forth in a Work Order. Base study fee is exclusive of analytical and bioanalytical chemistry support and toxicokinetic evaluation. This quotation is valid for 90 days with respect to authorization of the study, provided the study is initiated within six months from the date of this outline; thereafter the study fee is subject to review.
2. A minimum of 20 litters per group is recommended in this guideline.
3. Studies that do not establish a maternal NOAEL may be acceptable under this guideline.
4. These fees are considered estimates until the method has been developed. The fee for method development and validation is not included. Upon completion of the method development, the sponsor will be notified if different analysis fees apply. The costs also assume typical sample processing as well as standard analytical detection will be sufficient. Long processing procedures, long analytical run times, and mass spectrometric detection will result in an increased fee.
5. These fees are considered estimates. Additional samples and dilution repeats beyond 10% will be charged at a rate of $85/sample. The Sponsor will be notified in writing, prior to application of any such fees.
6. A report fee will be waived if there are ≥ 150 samples analyzed.

Fee and Payment Schedule:
20% upon signature of the Proposal
40% 45 days prior to animal arrival
30% upon completion of in-life
10% upon issuance of Draft Report

Sponsor Number:_____

Study Monitor/ Company Contact: _____ Purchase Order No. (if applicable):_____

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WIL RESEARCH

Analytical Validation, Homogeneity, and Stability Study of the Analyte in Aqueous Formulations
Compliance: GLP

Development and validation of a method for the determination of analyte concentration in aqueous formulations:

Method development usually includes (but is not limited to) the following activities: (1) investigation of potential solubility limitations; (2) the analysis of standards prepared in an appropriate solvent to establish chromatography, including retention times, resolution, and to check proportionality of response; (3) the analysis of the analyte prepared in the matrix to confirm the presence or absence of interferences, to evaluate potential stability limitations, and to evaluate response proportionality. Method development will be billed at a rate of $260/hour and will not exceed the amount proposed without sponsor approval.

Validation will be conducted using the current WIL SOP guidelines for the assessment of system suitability, method specificity/selectivity, intra- and inter-session method calibration acceptability, intra- and inter-session method accuracy and precision, ruggedness, and processed sample stability. A minimum of three validation sessions will be conducted. All laboratory work associated with validations will be conducted in accordance with applicable GLP regulations.

Homogeneity and stability assessment of analyte in aqueous formulations:

Testing includes the assessment of test article homogeneity in formulations spanning the range of concentration anticipated on future studies. In addition, resuspension homogeneity and stability will be assessed following a single storage duration. Additional stability time-points can be added for an additional fee. All laboratory work associated with sample analysis will be conducted in accordance with applicable GLP regulations.

Quality Assurance: The study will be conducted in compliance with Good Laboratory Practice (GLP) standards and will be monitored by the Quality Assurance Unit.

Reports: Audited Draft Report and Final Report.

Archiving: For a period of six months after study completion.

Summary of Fees:

Method Development (up to 16 hours):	$4,160
Method Validation in Aqueous Formulations: [2]	$11,000
Homogeneity and Stability Assessments in Aqueous Formulations: [2]	$6,800
Analytical Report:	$2,200
Base Fee [1]	$24,160

1. Final price depends on the technical details in the final protocol and will be set forth in a Work Order. This quotation is valid for 90 days with respect to authorization of the study, provided the study is initiated within six months from the date of this outline; thereafter the study fee is subject to review.
2. These fees are considered estimates until the method has been developed. Upon completion of the method development, the sponsor will be notified if different analysis fees apply. The costs also assume that typical sample processing as well as standard analytical detection will be sufficient. Long processing procedures, long analytical run times, and mass spectrometric detection will result in an increased fee.

Fee and Payment Schedule:
50% upon signature of the Proposal
40% upon completion of analysis
10% upon Issuance of Draft Report

Sponsor Number:_____

Study Monitor/ Company Contact: _____ Purchase Order No. (if applicable):_____

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WIL RESEARCH

Development and Testing of an LC-MS/MS Method for the Quantification of Test Article (TCE) and a Major Metabolite (TCA) in Rat Plasma
Compliance: Non-GLP

Development:	A fit-for-purpose LC-MS/MS method will be developed for the quantification of test article and one major metabolite in rat plasma. Appropriate chromatographic, mass spectrometric, and sample extraction procedures will be developed to achieve the sensitivity and specifications needed to support non-clinical studies of the test article.
Testing:	Once a suitable method has been developed, testing will be conducted that will include quantifying standards, quality control samples, and blanks to estimate the sensitivity, linearity, accuracy, and reproducibility of the procedure, and to ensure that the proper concentration range and conditions are selected prior to validation or analysis of study samples (as applicable). WIL Research will provide the Sponsor with timely updates on progress.
Quality Assurance:	The study will not be monitored or audited by the Quality Assurance Unit.
Archiving:	For a period of six months after study completion.

Summary of Fees:

Development and Testing [1,2,3]:	
24 hours @ $270/hour:	$6,480
Pre-Validation Testing 16 hours @ $270/hour:	$4,320
Materials:	$250
Base Study Fee [4]	$11,050

1. Method development and pre-validation will be billed at a rate of $270/hr. These activities will not be audited.
2. Species-specific plasma will be purchased from commercial sources and will be used as the blank (control) matrix. Estimated cost includes up to 100 mL of rat plasma.
3. The Sponsor will supply or reimburse for the test article(s) and suitable internal standard(s) (all with % purity ≥ 98%). Surcharges may apply for supplies that run outside the normal budget for this work.
4. Final price depends on the technical challenges encountered; additional time beyond that estimated above may be required; the Sponsor will be contacted for approval of any additional work. This quotation is valid for 90 days with respect to authorization of the study, provided the study is initiated within six months from the date of this outline; thereafter the study fee is subject to review.

Fee and Payment Schedule:
50% upon signature of the Proposal
50% upon completion of analysis

Sponsor Number:_____

Study Monitor/ Company Contact: _____ Purchase Order No. (if applicable):_____

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WIL RESEARCH

Validation of an LC-MS/MS Method for the Quantification of Test Article in Rat Plasma
Compliance: GLP

Validation:	Validation will be performed according to the FDA "Guidelines for Bioanalytical Method Validation" and 21 CFR Part 58, Good Laboratory Practice for Non-Clinical Laboratory Studies (revised as of April 1, 2007). Testing will include a minimum of 3 runs of matrix standard curves, along with at least 4 QC concentrations (LLOQ, low, medium, high) and at least 18 replicates total at each concentration. Intra-assay and inter-assay precision and accuracy of the QC samples will be determined. Validation will also include evaluation of linearity and limit of quantification, reproducibility, dilution effect, recovery, selectivity, carryover and processing, freeze-thaw, whole blood, and stock solution stability.
Stability:	All plasma stability evaluations will be performed at the low, high, and dilution QC levels. Long-term frozen storage stability testing at one time point and at one temperature is included in the validation fee.
Additional Fees:	Additional fees, $4,500/occasion, may be applied if additional stability time points/temperatures are requested by the Sponsor.
Protocol:	A protocol will be prepared by WIL Research for the validation. The Sponsor and/or Sponsor's representative will review the draft protocol and approve the final protocol.
Quality Assurance:	The study will be conducted in compliance with Good Laboratory Practice (GLP) standards and will be monitored by the Quality Assurance Unit.
Reports:	An audited draft validation report will be prepared by WIL Research and the Sponsor will be given time to review and comment on the report before it is finalized. The final bioanalytical procedure will be provided with the validation report. Requests for specific formatting for protocols and/or reports or multiple revisions may incur additional fees.
Archiving:	For a period of six months after study completion.

Summary of Fees:

Validation for Quantification [1,2,3]

Validation:	$28,000
Additional stability time points @ $4,500/time point:	TBD
Materials:	$750
Base Study Fee [4]	$28,750

1. The validation fee assumes quantification of a single analyte and is considered an estimate until the method has been developed. Upon completion of the method development, the sponsor will be notified if the base study fee will change. The final validation fee is dependent upon, but not limited to the suitability of the IS compound, LC run time, complexity and number of extractions, and other compound-specific issues.
2. Species-specific plasma will be purchased from commercial sources and will be used as the blank (control) matrix for assay validation and stability assessments as well as calibration and quality control sample preparation. Estimated cost includes up to 300 mL of rat plasma.
3. The Sponsor will supply or reimburse for the test article(s) and suitable internal standard(s) (all with % purity ≥ 98%). Surcharges may apply for supplies that run outside the normal budget for this work.
4. Final price depends on the technical details in the final protocol and will be set forth in a Work Order. This quotation is valid for 90 days with respect to authorization of the study, provided the study is initiated within six months from the date of this outline; thereafter the study fee is subject to review.

Fee and Payment Schedule:
50% upon signature of the Proposal
40% upon completion of analysis
10% upon issuance of Draft Report

Sponsor Number:_____

Study Monitor/ Company Contact: _____ Purchase Order No. (if applicable):_____

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WIL Research Laboratories LLC General Terms and Conditions

1. SERVICES AND COMPLIANCE. WIL will use commercially reasonable efforts to perform specific authorized services or studies ("Services") as set forth in the Proposal. WIL will comply with all laws, rules and regulations (collectively, "Laws") applicable to the Services performed. If any Laws change while Services are being performed, and such Laws, in WIL's reasonable judgment, necessitate a change in the Proposal, (a) WIL will submit to Sponsor a revised Proposal for Sponsor's review and acceptance prior to making any changes to Services and (b) WIL will not be required to perform any Service to the extent such performance would, in WIL's reasonable judgment, be in violation of a Law. In the event of a conflict between any applicable Laws, the Parties will mutually agree in writing as to the applicable Laws to be followed in WIL's performance of the Services. Sponsor will comply fully with all Laws applicable to the subject matter of the Services. Notwithstanding anything to the contrary contained herein, WIL may use one or more of its affiliates to perform the Services.

2. MODIFICATIONS. Sponsor will provide to WIL in writing any requested change to Services, and no such request, change, extension, revision or other modification to the Services or any Proposal will be binding unless agreed to in writing by the Parties.

3. COMPENSATION. The amount of all fees and expenses associated with the delivery to Sponsor of the Services are set forth in the Proposal. Sponsor will bear all taxes, fees and expenses other than those set forth in the Proposal. Invoices will be rendered in United States Dollars and provide for payment net 30 days. All invoices will be sent to Sponsor's address indicated in the Proposal, unless otherwise agreed to in writing by the Parties. WIL may request to increase the fees or expenses set forth in the Proposal to reflect any actual increase to its expenses incurred in connection with providing the Services. No such increase will be binding until consented to in writing by Sponsor, which such consent will not be unreasonably withheld. If Sponsor fails to pay an invoice within 45 days of its issuance date, WIL may, in its sole discretion, charge the Sponsor a late fee equal to 1.5% per month on the unpaid balance of such invoice until paid in full (including any assessed late fees) or treat such non-payment as notice by Sponsor to terminate the Services.

4. TERMINATION. (a) A Proposal or specific Services may be terminated as follows: (i) Sponsor may, at any time upon written notice to WIL, terminate the Proposal or specific Services for convenience. Such written notice must state the extent and the effective date of termination. Upon receipt of such notice, WIL will use commercially reasonable efforts to minimize costs to Sponsor resulting from such termination. (ii) WIL may terminate a Proposal or specific Services upon notice to Sponsor of Sponsor's breach or failure to perform any obligations required by this Agreement, including Sponsor's failure to cure payment default within 45 days of invoice issuance. (iii) Either Party may terminate any Proposal upon 90 days' prior written notice to the other Party. (iv) either Party may terminate a Proposal or specific Services upon 30 days written notice if any episode of force majeure described in Section 10 continues for 30 or more days after notification from the other Party of such episode. (b) If Services or Proposal are terminated for any reason pursuant to this Section 4, Sponsor will pay to WIL: (i) all amounts for authorized Services rendered through the effective date of termination; (ii) all wind-down costs incurred by WIL resulting from such termination; and (iii) all of WIL's costs and expenses incurred in preparation for providing the Services, including those incurred prior to commencement of authorized Services and whether invoiced or not. (c) Sponsor may, at any time upon written notice to WIL, delay authorized Services. Sponsor will pay WIL's costs and expenses incurred related to any such delay, and WIL will use commercially reasonable efforts to mitigate such costs and expenses until WIL receives written notice to resume performance of Services. (d) These General Terms and Conditions will apply to any Services performed pursuant to the Proposal, notwithstanding that the Proposal has been terminated, and will terminate upon completion of all outstanding Services, unless otherwise agreed to in writing by the Parties.

5. SURVIVAL. Notwithstanding the termination of the Proposal or specific Services thereunder, Sections 3 (Compensation), 4 (Termination), 5 (Survival), 6 (Intellectual Property & Work Product), 9 (Indemnification & Limiting Liability), 11 (Governing Law & Jurisdiction) and 13 (Miscellaneous) of these General Terms and Conditions will survive, unless otherwise agreed to in writing by the Parties.

6. INTELLECTUAL PROPERTY & WORK PRODUCT. Subject to the last sentence of this Section 6, all information or data collected, and all discoveries, inventions or improvements, whether patentable or not, other than WIL IP (as defined below), arising out of the performance of Services and relating to the articles or substances studied or the use thereof will be owned by Sponsor ("Sponsor IP"). At the request and sole expense of Sponsor, WIL will assign to Sponsor any and all of WIL's right, title and interest in Sponsor IP. Sponsor has no property rights in WIL's testing methods, practices, procedures, tests, test apparatus, equipment or information related to the conduct of WIL's business, or any inventions, improvements or developments related thereto ("WIL IP"). As between the Parties, WIL IP is the sole and exclusive property of WIL. Upon payment in full by Sponsor for all amounts invoiced hereunder, all tissues, tissue blocks, specimens, slides, material and data prepared or generated by WIL in the course of performing Services for Sponsor hereunder ("Work Product") will be owned by Sponsor and will be transferred to Sponsor upon its request after payment of such amounts.

7. INDEPENDENT CONTRACTOR. WIL is an independent contractor and that no provision in the Proposal, or any agreement subject to these General Terms and Conditions, will be construed to make WIL an employee, agent or representative of Sponsor, or be deemed to create a partnership or joint venture between the Parties. Neither Party will hold itself out to third persons as purporting to act on behalf of or serving as the agent of, the other Party.

8. WARRANTY. Other than as specifically set forth in Section 1, WIL makes no representations or warranties concerning the Services.

9. INDEMNIFICATION & LIMITING LIABILITY. WIL will indemnify, defend and hold harmless Sponsor, its directors, officers, equityholders and employees ("Sponsor Indemnitees") from and against all third party loss or damage (including

reasonable attorney fees and expenses) arising from (a) WIL's material breach of this Agreement or (b) WIL's negligence or willful misconduct in the performance of the Services, except to the extent such loss or damage arises from the negligence or willful misconduct of a Sponsor Indemnitee or Sponsor's material breach of the Agreement. Sponsor will indemnify, defend and hold harmless WIL and its affiliates and their respective directors, officers, equityholders and employees ("WIL Indemnitees") from and against all third party loss or damage (including reasonable attorney fees and expenses) arising from (a) Sponsor's material breach of this Agreement, (b) Sponsor's negligence or willful misconduct or (c) Sponsor's use or exploitation of any Sponsor IP, Work Product or Sponsor Confidential Information, except to the extent such loss or damage arises from the negligence or willful misconduct of a WIL Indemnitee or WIL's material breach of this Agreement. Under no circumstances will either Party be liable to the other for any indirect, consequential, punitive, exemplary or special damages, including lost profits or cost of replacement materials. Subject to any limitations on remedies set forth herein, in no event will WIL be liable to Sponsor under this Agreement for any amounts in excess of the amount paid by Sponsor to WIL for Services provided hereunder. If WIL commits a deviation during the performance of Services that causes the results of such Services to be unusable for Sponsor's stated purposes as defined in the relevant Protocol, then at Sponsor's election, WIL will either (i) rerun that part of the Services affected by such deviation or (ii) refund to Sponsor the sums paid WIL as of that date with respect to such Services. The remedies provided in the immediately foregoing sentence are the Sponsor's (and the other Sponsor Indemnitees') sole and exclusive remedy with respect to WIL's deviations in the performance of Services. The remedies provided in this Section 9 are the sole and exclusive remedies available to the Sponsor Indemnitees with respect to any breach of any representation, warranty or agreement in the Proposal, or otherwise in respect of the Services contemplated by the Proposal (whether in contract, tort, strict liability or otherwise).

10. FORCE MAJEURE. Neither Party will be liable for any delay in performing its obligations (other than payment obligations) under the Proposal if its performance is delayed or prevented by acts of God, fire, terrorist acts, explosion, war, riots, strikes, law or any other cause (except financial) beyond such Party's reasonable control, but only to the extent of such disability. If performance required by the Proposal fails during or subsequent to the occurrence of a force majeure event, performance will be deferred for a period of time equal to the period of disability resulting from force majeure.

11. GOVERNING LAW; JURISDICTION. This Agreement will be construed in accordance with and governed by the laws of the State of Ohio (without regard to any choice or conflicts of law rules that would cause the application of the laws of any other jurisdiction). The Parties irrevocably submit to the personal jurisdiction of the state and federal courts of the State of Ohio, and agree that such courts are the appropriate, exclusive and convenient forum for, and will have exclusive jurisdiction over, any action or dispute arising out of this Agreement or relating to any of the Services, and the Parties irrevocably waive any right to claim that such forum is inconvenient. Neither Party will bring suit with respect to any action or dispute arising out of this Agreement or relating to any of the Services in any court or jurisdiction other than the above specified courts. The preceding sentence will not limit the rights of the Parties to obtain execution of a judgment in any other jurisdiction.

12. ASSIGNMENT. The Proposal subject to these General Terms and Conditions, and any performance thereunder, constitutes a personal services contract and may not be assigned by either Party without the express written consent of the other, which consent may not be unreasonably withheld, except that either Party may assign this contract without consent in connection with a transaction resulting in (a) a change of control with respect to such Party or (b) the acquisition of all or substantially all of such Party's assets by such assignee.

13. MISCELLANEOUS. [Insurance] WIL will maintain in full force and effect during the performance of Services, a policy or policies of insurance commensurate with industry standards for services substantially similar to the Services performed by WIL. [Delivery and Transfer] Any materials or Work Product shipped to WIL by Sponsor or a third party, or shipped by WIL to Sponsor or to a third Party, shall be at Sponsors expense. Therefore, Sponsor will pay any shipping or transportation costs and taxes, including any import or export duties, fees, and taxes. All Work Product will be appropriately packaged and labeled pursuant to WIL's standard operating procedures and delivered to a common carrier for shipment. Sponsor will hold WIL harmless from and against all loss or damage or claims of loss or damage to any Work Product during shipment by a common carrier. Sponsor will also pay the insurance premium and will notify WIL, in writing, of its desire to insure shipments at a rate that exceeds the common carrier's standard liability limit. In the event a claim results, Sponsor shall be responsible for substantiating (if required by the insurer) the value of the Work Product and for seeking reimbursement of any loss. [Severability] If a court of competent jurisdiction finds a provision of these General Terms and Conditions, the Proposal, or any agreement between the Parties subject hereto, to be invalid or contrary to public policy, the provisions not so found will remain in effect and binding upon the Parties. The Parties will agree in good faith to replace any invalid or unenforceable provision with a valid and enforceable provision that expresses as closely as possible the intention of the original provision. [Publications] Neither Party will use the name of the other Party or the other Party's employees in any advertising, sales promotional material, or in any publication without such other Party's prior written consent. [Dispute Resolution] The Parties will attempt in good faith to resolve any dispute arising hereunder prior to taking any legal action. If Parties are unable to resolve any such dispute within 30 days, each Party may seek any legal remedy available in accordance with these General Terms and Conditions. Notwithstanding the foregoing, either Party may seek interim legal relief in a court of competent jurisdiction if the other Party's breach of their obligations under any agreement subject hereto would reasonably be expected to cause such Party irreparable harm. [Precedence] No modification or waiver of the provisions of these General Terms and Conditions shall be valid or binding on either Party unless in writing and signed by both Parties. Unless otherwise expressly agreed to in writing by the Parties, in the event a Proposal, Protocol, or any other agreement between the Parties hereto conflict with or contradict these General Terms and Conditions, then these General Terms and Conditions shall control. [Counterparts] Any agreement between the Parties related to the Services (including any Proposal) may be executed in counterparts, each of which will be deemed an original, and all of which together will be deemed to be one and the same instrument. Signatures

to any agreement between the Parties related to the Services transmitted by facsimile transmission, by electronic mail in "portable document format" (".pdf") or similar form or by any other electronic means (e.g. DocuSign) intended to preserve the original graphic and pictorial appearance of a document will have the same effect as physical delivery of the paper document bearing the original signatures, and will be deemed original signatures by both Parties.

[Remainder of left blank]

WIL RESEARCH

WIL RESEARCH LABORATORIES LLC CANCELLATION AND DELAY POLICY

Timing	Cancellation Fee	Delay Fee
More than 45 days prior to animal arrival.	10% of the total fee under the signed proposal.	No fee.
Less than 45 days prior to animal arrival.	20% of the total fee under the signed proposal, plus Costs Incurred (as defined below).	$2,100 per day for each room utilized
Any time after animal receipt.	50% of the total fee under the signed proposal, plus the cost of any animals ordered under the proposal and any Costs Incurred.	$2,100 per day for each room utilized plus any Costs Incurred.
Non-animal related studies.	Costs Incurred, for study preparation and conduct including but not limited to time and materials related to protocol preparation and protocol activities, instrument set up, study termination, and reporting (if required)	No fee.

- Unless otherwise expressly agreed to in writing by the Parties, the fees and obligations detailed in this policy are in addition to the written terms and conditions, or any other agreement, as may be agreed to by the Parties.
- Actual fees may vary depending on the nature and specifications of the services (*e.g.* Costs Incurred, species, the number of animals involved, unique animal specifications).
- WIL Research Laboratories LLC ("WIL") shall, in good faith, use commercially reasonable efforts to mitigate costs incurred resulting from any cancellation or delay.
- Upon Sponsor's request, WIL shall make a good faith effort to reschedule cancelled or delayed services as close as possible to the requested time frame.
- Cost Incurred may (i) prior to commencement of services include any reasonable costs and expenses related to study preparation, time and materials related to protocol development, (ii) following cancelation or delay include any reasonable costs and expenses related to maintenance of animals or materials, reoccurring costs related to such delay, any reporting (if required), and any wind-down costs resulting from such cancellation or delay (*e.g.* necropsy). Additionally, in each case, if large animals were ordered or used, then Costs Incurred shall also include the cost to maintain such large animals which such cost will not be less than $2,100 per day for each room utilized, for a minimum of 30 days.
- This information is provided at the request of Sponsor and is intended for the sole use of Sponsor in regards to the services provided by WIL. Further, this information is considered confidential and is not to be copied or shared with any third party unless approved in writing by WIL prior to any disclosure.